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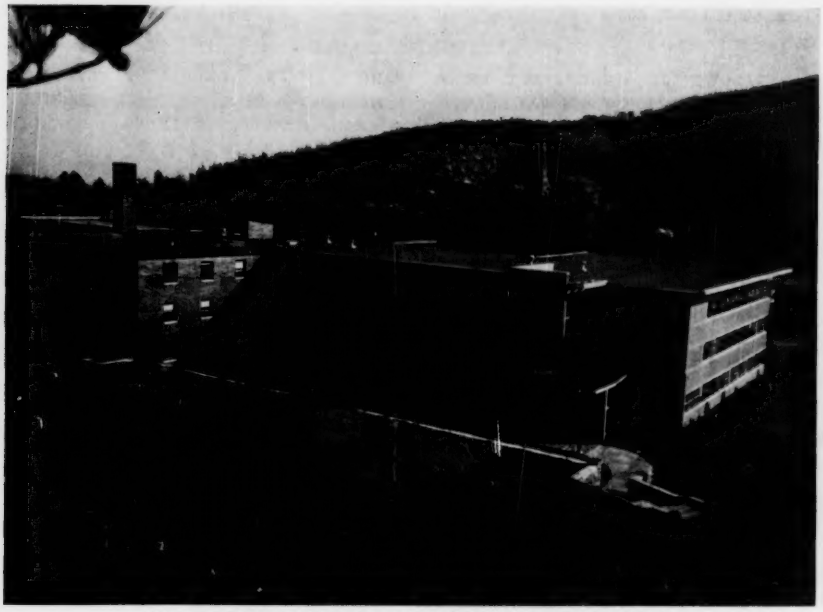

THE ROSCOE B. JACKSON MEMORIAL LABORATORY

JACKSON LABORATORY'S THIRTIETH ANNIVERSARY—
A SPECIAL ISSUE

Hepatic Fusion

Neonatal Jaundice

Longevity of a Lethal

"Wuzzy" Mutation in the Rabbit

A New Linkage Map of the Mouse

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CONTENTS

LINKAGE MAP OF THE MOUSE
By Margaret C. Green and Margaret M. Dickie 3

VARIABILITY IN ACTIVITY WITHIN INBRED AND CROSSBRED MICE
By Arnold M. Mordkoff and John L. Fuller 6

VISCERAL INVERSION AND ASSOCIATED ANOMALIES IN THE MOUSE
By Katharine P. Hummel and Dorothy B. Chapman 9

SUBSPECIFIC DIFFERENCES IN THE RESPONSES OF YOUNG DEERMICE ON AN ELEVATED MAZE
By John A. King and Nancy J. Shea 14

SELECTION AND INBREEDING FOR LONGEVITY OF A LETHAL TYPE
By Elizabeth S. Russell and Fay A. Lawson 19

RATES OF SUBLINE DIVERGENCE IN HIGHLY INBRED STRAINS OF MICE
By Donald W. Bailey 26

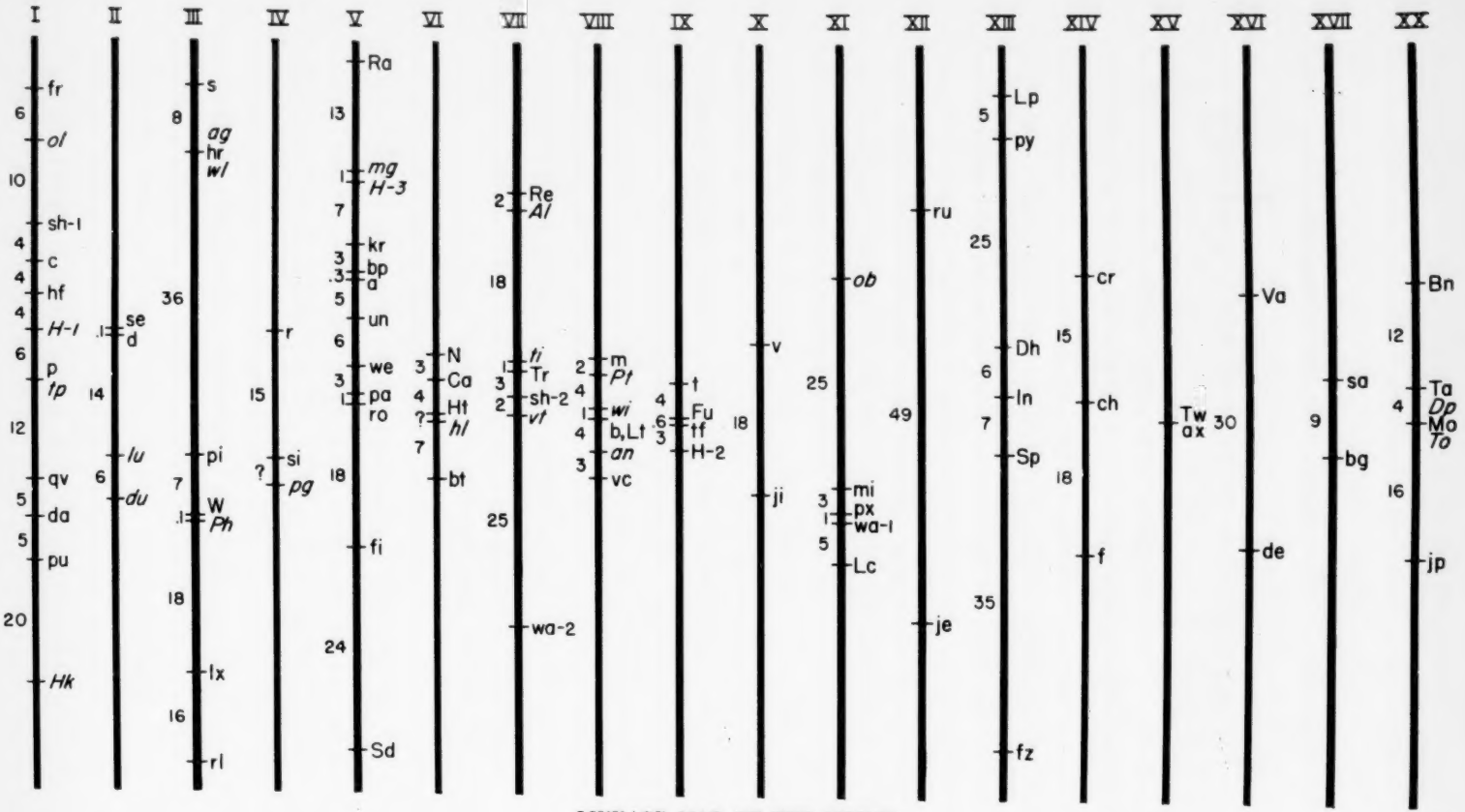
INHERITANCE AND HAIR MORPHOLOGY OF THE "WUZZY" MUTATION IN THE RABBIT
By D. D. Crary and P. B. Sawin 31

A MUTATION CAUSING NEONATAL JAUNDICE IN THE HOUSE MOUSE
Leroy C. Stevens, Judith A. Mackensen and Seldon E. Bernstein 35

HEPATIC FUSION, A NEW GENE IN LINKAGE GROUP I OF THE MOUSE
By Lester E. Bunker, Jr. 40

Notes and Reviews

GENETIC FACTORS IN PHYSIOLOGICAL ADAPTATION
By Wm. G. Downs, Jr., G. B. Pennybaker and Mel Bowling 5



LINKAGE MAP OF THE MOUSE

Figure 1

LINKAGE MAP OF THE MOUSE

MARGARET C. GREEN AND MARGARET M. DICKIE*

THIS map summarizes the most recent information on linkage in the house mouse. Most of it is based on published material but it also makes use of information from personal communications and from Mouse News Letter (a mimeographed bulletin distributed to research workers interested in mice). The unpublished information is used by permission of the authors.

Mutant genes have been included or excluded from the map on the basis of the following criteria:

1. No genes known to be extinct have been included.

2. The numerous alleles at the histocompatibility loci and the *t* locus have not been listed.

3. Only linkages published before June 1958 or noted in Mouse News Letter through No. 19, July 1958, have been included. Further information on some of these linkages has been supplied by personal communication.

The distances between loci shown are recombination percentages. They are of varying degrees of reliability, that for the distance between *c* and *p* in linkage group I, for instance, being based on tests with about 24,000 mice, while for some others the number of mice classified is less than 100. The linear order of many of the genes is subject to some degree of uncertainty. Those indicated by symbols in italics have not been critically tested to determine their position relative to all other genes in the linkage group. The order of the other genes is established with a fairly high probability, but it would not be too surprising if further evidence showed some of these to be incorrectly placed. In particular, *ru* and *je* in linkage group XII show only very loose linkage or, in some bodies of data, none at all. They may therefore be in different linkage groups.

The names of the genes are given in the following list. The date following the number of each linkage group is the year the first linkage in that group was discovered.

I *1915*

fr	frizzy
ol	oligodactyly
sh-1	shaker-1
c-series	
c^{ch}	chinchilla
c^{e}	extreme dilution
c	albino
hf	hepatic fusion
H-1	Histocompatibility-1
p	pink-eyed dilution
tp	taupe
qv	quivering
da	dark
pu	pudgy
Hk	Hook

II *1927*

se	short ear
d series	
d	dilute
d^{l}	dilute-lethal
lu	luxoid
du	ducky

III *1931*

s	piebald
ag	agitans
hr series	
hr	hairless
hr^{rh}	rhino
wl	wabbler-lethal
pi	pirouette
W series	
W	Dominant spotting
W^{v}	Viable dominant spotting
W^{j}	Jay's dominant spotting
W^{a}	Ames dominant spotting

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Ph	Patch
lx	luxate
rl	reeler
	IV *1930*
r	rodless retina
si	silver
pg	pygmy
	V *1935*
Ra	Ragged
mg	mahogany
H-3	Histocompatibility-3
kr	kreisler
bp	brachypodism
a series	
A^y	Yellow
A^w	White-bellied agouti
a^t	black and tan
a	non-agouti
a^e	extreme non-agouti
un	undulated
we	wellhaarig
pa	pallid
ro	rough
fi	fidget
Sd	Danforth's short tail
	VI *1939*
N	Naked
Ca	Caracul
Ht	Hightail
hl	hair-loss
bt	belted
	VII *1939*
Re	Rex
Al	Alopecia
ti	tipsy
Tr	Trembler
sh-2	shaker-2
vt	vestigial tail
wa-2	waved-2
	VIII *1942*
m	misty
Pt	Pintail
wi	whirler
b series	
b^e	cordovan
b	brown
Lt	Light
an	anemia
vc	vacillans
	IX *1942*
T series	
T	Brachyury
t	tailless
Fu series	
Fu	Fused
Fu^{ki}	Kinky
tf	tufted
H-2	Histocompatibility-2
	X *1945*
v	waltzer
ji	jittery
	XI *1948*
ob	obese
mi series	
Mi^{wh}	White
mi	microphthalmia
px	postaxial hemimelia
wa-1	waved-1
Lc	Lurcher
	XII *1948*
ru	ruby eye
je	jerker
	XIII *1953*
Lp	Loop tail
py	polydactyly
Dh	Dominant hemimelia
ln	leaden
Sp	Splotch
fz	fuzzy
	XIV *1953*
cr	crinkled
ch	congenital hydrocephalus
f	flexed tail
	XV *1956*
Tw	Twirler
ax	ataxia
	XVI *1957*
Va	Varitint-waddler
de	droopy-ear
	XVII *1958*
sa	satin

bg	beige	*Dp*	Dappled
	XX *1952*	*Mo* series	
	Sex-linked	*Mo*	Mottled
Bn	Bent	Mo^{br}	Brindled
Ta	Tabby	*To*	Tortoise
		jp	jimpy

GENETIC FACTORS IN PHYSIOLOGICAL ADAPTATION

STUDIES on the adaptive response of laboratory animals (different strains of calves, mice and rats)to various kinds of "stress," i.e., insulin, ACTH, severe cold, have elicited some interesting facts of genetic importance. Response to stress is measured by total and differential leucocyte counts and rectal temperatures, taken at hourly intervals after injection or application of physical stress for a total eight-hour period. All animals have previously had complete eight-hour observations made, covering the same time period (12 noon to 9:00 P.M.) these charts serving as a control basis.

In many cases, even where the animals had been "inbred" for several generations, there were marked differences in the "normal" picture of littermates of the same sex. Not infrequently these differences were as great as between control and experimental results.

Only when breeding animals are selected for close similarity in blood-picture at similar diurnal periods and are bred brothert-o-sister for a minimum of three generations to a point of being homozygous for blood-count, are control stocks of value for such studies. Such stocks are now being used in this laboratory, and are giving origin to quite consistent experimental results.

A marked difference is also noted in response to stress as between sexes, even when these are littermates out of homozygous (for blood-count) stocks. An effort is being made to analyze the genetic factors concerned in these mechanisms.

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VARIABILITY IN ACTIVITY WITHIN INBRED AND CROSSBRED MICE

A Study in Behavior Genetics

ARNOLD M. MORDKOFF AND JOHN L. FULLER*

GRÜNEBERG[5] has summarized evidence on morphological variability of inbred strains and their F_1 hybrids. Experiments on physiological variability have dealt with time of vaginal opening[11], response to Nembutal[7], and response to androgen[1]. These investigations indicated that inbred strains of mice exhibit greater phenotypic variability than do their F_1 hybrids. The regulatory process which leads to constancy of phenotype has been described as existential adaptation[3], canalization[9], homeostasis[2] and developmental homeostasis[6]. The connection between this phenomenon and heterosis have been recognized.

Both developmental homeostasis and enhanced vigor have been ascribed to heterozygosity. Thus it is argued that a decrease of phenotypic variability is not a necessary consequence of the decreased genetic variability produced by inbreeding. If the ability to buffer environmental stresses is reduced in homozygous individuals, phenotypic variability might even increase. It should be recognized that the concept of developmental homeostasis seems to have little merit when one deals with biochemical characters which are closely dependent upon the primary action of genes. Its application is to the end product of long chains of events during which environmental factors have many opportunities to modify the pattern. Hence the idea seems particularly applicable to behavior genetics, for behavior is a series of organism-environment interactions, in which the later members of the series are clearly influenced by earlier members. In other words, behavior is modified by previous experience.

Despite the pertinence of the theory to behavior genetics we know of no direct tests using behavior measures. To provide such a test was the objective of the experiment to be described. Activity was chosen as the parameter to be studied because it can be measured quantitatively, and because it is a form of behavior involved in many kinds of psychological experimentation. For example, differences in activity may affect maze learning or success in tests involving manipulation of objects. An activity test was administered repeatedly to individuals from two inbred strains of mice, to their F_1 hybrid, and to a relatively random bred strain in whose ancestry the two inbred strains were represented. The experimental design was planned to measure two kinds of variability, (1) subject variability within strains, and (2) trial to trial variability within subjects. The inbred strains and their F_1 hybrid were genetically homogeneous, while the random bred strain was heterogeneous. Individuals of the F_1 and random bred strain were relatively heterozygous, as compared with the highly homozygous individuals of the inbred strains. If heterozygosity contributes to developmental homeostasis, the subject to subject variability of the inbred strains might be higher than the F_1, and perhaps comparable to the random bred strain. One might also predict that both random bred and F_1 individuals would be more consistent from trial to trial.

Materials and Methods

The subjects were 80 male mice of the following types: 20 C57BL/6, 20 DBA/2, 20 BDF_1 (hybrid of C57BL/6 females and DBA/2 males), and 20 DX (progeny resulting from random mating of double-cross hybrids of C57BL/6, DBA/2, BALB/c, and

*Wesleyan University, Middletown, Connecticut and Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The authors gratefully acknowledge the support of the Ford Foundation, and the kindness of Dr. E. L. Green in providing the DX mice.

C3HeB inbred strains). Each mating which produced the double-cross hybrid was made so that all four lines were represented in the parentage. All animals were obtained from the colony at the Jackson Memorial Laboratory. They were six weeks old on the first day of testing except for the DBA/2's which were a week younger.

The animals were housed in standard mouse cages 12 × 12 inches divided into two pens, from three days prior to the start of the experiment to its completion. Two to three animals were housed in each pen. Food and water were provided *ad libitum* throughout the experiment.

The apparatus consisted of four runways, 3 feet long and 4 inches wide, equipped with metal covers 4 inches high. There were three treadles on each runway connected via microswitches to Veeder Root Magnetic Reset Counters which were in a room adjoining the experimental chamber. Each subject was treated to one 10-minute trial in each runway on four successive days.

TABLE I. Means, standard deviations, and coefficients of variation for activity scores of individuals

Strain	Mean	S.D.	C.V.	S.E.C.V.
DX	22.7	10.1	44.5	4.1
BDF_1	13.4	6.3	47.0	4.4
C57BL	25.1	15.0	60.0	6.2
DBA	10.9	5.4	50.0	4.8

Results

When placed in the runways most mice responded by a period of exploration. Activity gradually subsided during the course of the 10-minute trial. The most active group, C57BL contacted over twice as many treadles on the average as the least active DBA (Table I). The high standard deviations indicate considerable individual variability, even among highly uniform subjects.

The statistical comparisons with respect to variability are summarized in Table II. The sum of squares between subjects within groups is the index of subject variability. The sum of squares of the trial by subject interaction is a measure of trial variability for individuals.

The Hartley F max test was used to test significance of heterogeneity of variance between groups. Individual F's are given in Table III.

Ranked with respect to between-subject variability the order from highest to lowest was C57BL, DX, BDF_1 and DBA. The two lowest were not significantly different; the

TABLE II. Analysis of variance of activity of inbred, hybrid, and random bred mice

Source of variation	Sum of squares	Df	Mean squares
Between groups	11,493	3	3,831
Between Ss within groups	31,292	76	869.2
a) C57BL/6	17,716	19	932.4
b) BDF_1	3,153	19	165.9
c) DX	8,101	19	426.4
d) DBA/2	2,322	19	122.2
Total between subjects	42,785	3	
Between trials	4,953	79	1,651.0
Trials × groups (interaction)	1,856	9	206.3
Trials × Ss within groups (interaction)	40,406	228	177.2
a) C57BL/6	11,912	76	156.7
b) BDF_1	7,305	76	96.1
c) DX	16,177	76	212.9
d) DBA/2	5,012	76	65.9
Total within subjects	47,215	239	
Total sum of squares	90,000	319	

TABLE III. F scores for heterogeneity of variance between groups for between and within subjects

	DX		BDF_1		C57BL	
	Between subjects	Within subjects	Between subjects	Within subjects	Between subjects	Within subjects
BDF_1	2.57*	2.21**†				
C57BL	2.19*	1.36	5.6**†	1.63*		
DBA	3.49**	3.22**	1.36	1.45	7.63**	2.38**

*Significant at .05 level
**Significant at .01 level
†Hartley F max Test

other differences were significant at the .05 level or better. Thus the two inbred strains were found at the extremes. The ranking with respect to trial to trial variability was DX, C57BL, BDF_1 and DBA. Again the hybrids have low variance, but the two inbred strains differ significantly.

The DBA group obviously did not behave in the fashion predicted by the hypothesis. There are two possibilities; (1) the hypothesis that uniform homozygous population are more variable than uniform heterozygous ones is invalid, or (2) not all assumptions were met in the DBA group and their behavior is not an appropriate test of the hypothesis. The DBA's were unavoidably tested one week younger than the other groups. Grüneberg[4] states that changes accompanying sexual maturity occur during the fifth week of life. These might produce a consistency not characteristic of the group at a later age, but this seems unlikely.

There is, however, a positive correlation between means and variances of the four groups. The smaller sums of squares of the DBA and BDF_1 could be merely a function of relative inactivity within the apparatus. Snedecor[8] states, "It seems rather characteristic that large things vary very much and small things little." Commonly, raw scores are transformed to compensate for this fact, and variances are made equal in order to test for the heterogeneity of means. Such a procedure would, of course, abolish the effect for which we were looking. Ideally all groups should have the same mean, but we have been unable to accomplish this by a logical transformation. As a substitute the standard deviations were expressed as fractions, coefficients of variation (CV), of the sample mean. When expressed in this fashion (Table I) the relative variability of the four group changes. Again the C57BL's exhibit most variability, and the others rank DBA, DBF_1 and DX. Standard errors have been calculated for the CVs, but no formula was found for an exact significance test.

Discussion

The purpose of this experiment was to inquire whether increased uniformity of behavior, specifically activity in the house mouse. The results are equivocal because of the correlation of means and variances. One might conclude that the genetic background had an effect upon activity, but no important effects upon variability. The random bred animals were surprisingly uniform from subject to subject despite genetic heterogeneity. There is no clear reason why they should have been most variable from trial to trial. The results raise a question as to whether the concept of developmental homeostasis has the same meaning for behavior as for morphology or physiological regulation. Individual animals do not have an opportunity to try several types of structural development and select the most adequate, yet this is exactly how behavior adjustment occurs in higher animals. Variability in behavior may actually provide better homeostasis than uniformity. This investigation was not extensive enough to test all possibilities.

The results of this experiment have some significance to the selection of subjects of minimum phenotypic variability for psychological experiments. Hybrids between inbred strains are an excellent choice, but the disadvantage of the inbred strains is not great. Further experiments are planned to control for the differences in mean activity by selecting inbred strains which are closer in phenotype and comparing them with their F_1 hybrid. Planned variation in the test situation will also be employed in order to discover whether genetics does play a role in trial to trial variability.

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VISCERAL INVERSION AND ASSOCIATED ANOMALIES IN THE MOUSE

KATHARINE P. HUMMEL AND DOROTHY B. CHAPMAN*

SITUS inversus viscerum has been described rarely in mice, and reports in the literature are limited almost exclusively to cases noted at autopsy[6]. Among 1,817 mice autopsied for visceral anomalies, Bagg[1] found only one with reversed viscera. Strong[11] found one among descendants of methylcholanthrene-treated mice and remarked that over a period of 25 years, he had observed only one other in a colony of 210,000 mice. Hummel[7] reported finding seven among 350 adults of the BALB/c strain autopsied, and none among mice of eight other inbred strains. Tihen, Charles and Sipple[12] found none in examining more than 11,500 hybrids of a cross between mice of strains C57BL and DBA, but in a stock constituted by crossing these two strains with the BALB/c and Swiss, they found animals in which the condition was inherited as an autosomal recessive with incomplete penetrance. In addition to left-right transposition of viscera and blood vessels, manifestations included abnormalities in spleen shape, lung lobation, and position of postcaval and hepatic portal veins. Mortality was high in affected animals; they were small, sickly-looking, and in many cases appeared to be hydrocephalic.

Since 1948, one more case of situs inversus viscerum has been found in a mouse of the BALB/c strain in our colony. The animal, a male identified as transposed at birth, was raised and bred, and many descendants, including backcrosses, were examined. Neither situs inversus nor other abnormalities were found and it was concluded that in this case, the condition was not genetic. At about the same time, two newborn mice with stomachs on the right, rather than the left, were noticed in a litter of 12, born to a pair (brother and sister) of mice in a non-inbred stock homozygous for myelencephalic blebs (*mymy*). This pair of mice had several more litters; in a total of 43 offspring examined, five with transposed viscera were found. At autopsy, the parents were found to be normal with respect to situs inversus. Three of the five survived, were bred and produced healthy offspring with transposed viscera and abnormalities of blood vessels, spleen, liver and lungs. Studies have shown that this syndrome of visceral inversion and accompanying abnormalities is inherited as a single autosomal recessive gene (symbol *iv*) with incomplete penetrance. Thus in its inheritance and expression, this mutation is similar to that described by Tihen and associates, but as their mutation is believed to be extinct, its identity with *iv* can not be tested.

Mice with reversed viscera can be identified as soon after birth as milk has been ingested and until they are four to five days old, after which time, the stomachs can not be seen readily through the thickened skin. Using stomach and also spleen position, mice were classified neonatally for visceral inversions; for the observation of other abnormalities not involving stomach position, autopsies were necessary.

Several types of matings were made. Mice with transposed viscera were mated together and with normal animals of strains DBA, BALB/c and C3H. The F_1 hybrids were mated together and backcrossed to "reversed" animals. In this way, a stock of mice homozygous for situs inversus was established. At

* Roscoe B. Jackson Memorial Laboratory, Bar Habor, Maine. This investigation was supported in part by a research grant RG 4636 from the National Institutes of Health, Public Health Service.

TABLE I. Classification by phenotype of mice homozygous for *iv*

	1		2		3		4		5		6		13		Total	unreversed
	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
♀	82	31	8	3	2	0.7	26	10	5	2	10	4	6	2	139	53
♂	63	26	4	2	3	1	20	8	2	0.8	19	8	2	0.8	113	46
Total	145	29	12	2	5	0.9	46	9	7	1	29	6	8	1.5	252	50
	7		8		9		10		11		12		14		Total	reversed
♀	66	25	10	4	3	1	21	8	5	2	18	7	1	0.3	124	47
♂	84	34	5	2	3	1	10	4	3	1	22	9	4	2	131	54
Total	150	30	15	3	6	1	31	6	8	1	40	8	5	0.9	255	50
	1+7		2+8		3+9		4+10		5+11		6+12		13+14		Total	
♀	148	56	18	7	5	2	47	18	10	4	28	11	7	3	263	52
♂	147	60	9	4	6	2	30	12	5	2	41	17	6	2	244	48
Total	295	58	27	5	11	2	77	15	15	3	69	14	13	3	507	—

the same time, there was selection against mice homozygous for myelencephalic blebs. Offspring of all matings were examined at birth, and were autopsied either neonatally, at weaning, or after their periods of reproductive usefulness. They were then classified according to the pattern of anomalies found.

Morphology

Asymmetrical structures observed to be anomalous in position or shape in the situs inversus mice are listed, and the normal as well as abnormal conditions described briefly.

Abdominal viscera

Liver: divided into median, caudate, and right and left lateral lobes. The median lobe is deeply notched and contains the gall bladder; the caudate lobe surrounds the oesophagus in the lesser curvature of the stomach; and the right lateral lobe is subdivided into anterior and posterior portions. The more caudal or posterior portion of the right lateral lobe lies medial to the anterior pole of the right kidney and receives the renal segment of the inferior vena cava.

Stomach: lies in the anterior left quadrant of the abdominal cavity with the forestomach on the left and the pylorus on the right.

Spleen: a flat, elongated oval body lying along the greater curvature of the stomach.

Kidneys: the left is posterior (caudal) to the right.

Thoracic viscera

Lungs: divided into five lobes, one from the left bronchus and four from the right, one of the latter being centrally located.

Heart: apex is on left, formed by the more muscular systemic ventricle; larger right atrium receives both of the superior venae cavae, as well as the hepatic segment of the inferior vena cava.

Veins

Superior venae cavae: right and left; the left is longer and passes dorsal to the base of the heart to reach the right atrium.

Azygos: located to the left of the aorta and vertebrae against the dorsal wall of the thoracic cavity; receives branches from both right and left intercostal spaces and joins the left superior vena cava. A hemiazygos vein, if present, collects from the right posterior intercostal spaces and joins the azygos in the 9th or 10th intercostal space.

Inferior vena cava: a single vessel lying to the right and slightly ventral to the aorta in the lumbar region. After receiving the left and right renal veins, it enters the posterior division of the right lateral lobe of the liver. It receives hepatic veins in the liver, emerges from the median lobe, pierces the diaphragm and as the hepatic segment traverses the thoracic cavity to enter the right atrium together

with the left superior vena cava.

Hepatic portal: formed by union of veins from the intestine, pancreas and spleen, it passes dorsal to the duodenum to enter the median lobe of the liver close to the gall bladder.

In mice with the genotype *iviv*, all of these structures were found transposed right to left in a mirror image. Both complete and partial reversals were found, and in addition, other anomalies were observed. In the liver, all or part of the caudate lobe might be missing, and the posterior subdivision of the right lateral lobe reduced or missing. In the latter case, the renal segment of the inferior vena cava did not enter the liver but continued by way of the azygos vein and superior vena cava to the right atrium, and the hepatic segment was attenuated.

Since the azygos vein and the inferior vena cava normally develop on opposite sides of the body, one or other must be in abnormal position for them to be continuous. Two situations were observed. The inferior caval and azygos veins were continuous on the right side as is normal for the inferior vena cava, or continuous on the left, the normal position of the azygos vein.

In the hepatic portal anomaly, the vein passed ventral to the duodenum on its way to the median lobe of the liver. Lung lobation varied from 1 lobe each side to 4 each side in various combinations which will not be listed in detail. The spleen varied in shape, and position, being thickened, shortened, constricted in the middle, dorsal to the stomach, or extending across the body.

As a rule, all of the viscera were transposed together, although in a few cases, abdominal and thoracic viscera were not concordant. Most of the animals fell into 12 categories with regard to transpositions of viscera and blood vessel patterns. In the first 6 of these, the abdominal viscera were not reversed, and the animals would have been marked as normal soon after birth. Animals of groups 7 through 12 had reversed stomachs which would have served to identify them at birth. Groups 13 and 14 included the few cases where the transpositions and blood vessels did not fall into specific patterns. The 14 categories used to classify mice of the *iviv* genotype are listed.

Viscera not reversed

1. Normal relationship, position and shape of all asymmetrical structures.
2. Like 1, but with the hepatic portal anomaly.
3. Inferior vena cava on left (abnormal), joining left azygos vein (normal).
4. Like 3, but with the hepatic portal anomaly.
5. Inferior vena cava on right (normal), joining right azygos vein (abnormal).
6. Like 5, but with hepatic portal anomaly.
13. Blood vessels in miscellaneous abnormal patterns.

Viscera reversed

7. Mirror image of 1. (complete "normal reversal").
8. Mirror image of 2; like 7, but with hepatic portal anomaly.
9. Mirror image of 3.
10. Mirror image of 4; like 9, but with hepatic portal anomaly.
11. Mirror image of 5.
12. Mirror image of 6; like 11, but with hepatic portal anomaly.
14. Blood vessels in miscellaneous abnormal patterns.

Abnormal lung and liver lobations, and abnormal spleen shapes and positions occurred among mice of all groups except 1 and 7.

In all, 507 mice born to parents with transposed viscera were autopsied and classified. Table I summarizes the observations for both males and females. From these data, it is apparent that only 50 percent of the mice would have been classified as situs inversus by observation of stomach position; autopsy showed that actually 71.4 percent of the population was affected. Only 145 of the 252 mice classified as "unreversed" proved on autopsy to be entirely normal in respect to situs inversus and associated anomalies. These 145 mice (group 1) were the normal overlaps and represented about one half of the "unreversed" animals and 28.6 percent of the *iviv* mice classified. A like proportion of the 255 mice with reversed viscera were mirror images of normal. These 150 mice (group 7), designated as complete "normal reversals," made up 29.6 percent of the observed population and 58.8 percent of the "reversed" animals.

The largest group (41.8 percent) was composed of the 212 mice of groups 2 through 6 and 8 through 14, with partial transpositions and blood vessel anomalies. In 172 or 81 percent of these 212 animals, the inferior vena cava by-passed the liver and joined the azygos; in 173 or 82 percent the hepatic portal

TABLE II. Incidences of abnormalities among mice homozygous for *iv*

Abnormality	No. Observed			Abnormal					
	♀	♂	Total	♀		♂		Total	
Transpositions	263	244	507	181	68.8%	181	72.2%	362	71.4%
Hepatic portal anomaly	263	244	507	93	35.4%	80	32.7%	173	34.1%
Postcava-azygos anomaly	263	244	507	90	34.2%	82	33.6%	172	33.9%
Lung lobation	270	247	526	56	20.7%	50	19.5%	106	20.1%
Spleen shape or position	272	247	519	78	28.6%	73	29.5%	151	29.0%

anomaly was present; and 146 mice or 69 percent had both of these anomalous patterns. Very few animals, on the other hand, had the inferior vena cava anomaly without the hepatic portal anomaly (26 mice or 12.6 percent) or the hepatic portal anomaly without the inferior vena cava anomaly (27 mice or 12.7 percent).

It is also noteworthy that among the 172 mice in which the azygos vein and superior vena cava served to return blood from the posterior part of the body to the heart, the azygos vein had developed in normal relationship to the aorta in approximately one half of the cases (88), and the inferior vena cava had developed normally in approximately one half (84).

Although it might appear that there were more "normal reversal" males than females, the difference is not significant ($P = 0.2\text{-}0.1$); in fact there were no differences in any of the groups in regard to sex.

Different numbers of mice were observed for abnormal spleens and abnormal lung lobations, as it was sometimes impossible to determine the course of blood vessels or shapes of spleens in animals which were found dead. In 526 offspring of animals with visceral inversion, 106 or 20 percent had abnormal patterns of lung lobation; in 519, 151 or 29 percent had spleens, abnormal in shape or in position relative to the greater curvature of the stomach. In neither group were there differences in incidence between males and females. These observations are summarized in Table II, and compared with incidences of visceral transposition and anomalous blood vessel pattern.

Inheritance

Observations on the penetrance of situs inversus in the different types of crosses are summarized in Table III. No mice with abnormalities were found in hybrids produced by crossing "reversed" animals (*iviv*) with normal animals (++) of the three inbred strains, BALB/c, DBA and $C3H_e$. When the F_1 hybrids were mated together ($iv+ \times iv+$), less than half of the expected number of abnormals were found in the F_2 generation, even after careful examination at autopsy. Although penetrance was higher in backcrosses ($iv+ \times iviv$) and in intercrosses ($iviv \times iviv$), it was still reduced. Normal phenotypes, derived from intercrosses between affected animals, when mated to "reversed" animals gave the same proportion of abnormal offspring as did matings between "reversed" animals, indicating that the phenotypically normal animals were indeed normal overlaps with the *iviv* genotype.

From these data, it was concluded that the visceral inversions and associated abnormalities are inherited as a single autosomal recessive character with incomplete penetrance.

Discussion

Whereas the situs inversus mice described by Tihen *et al.*[12] were small and sickly with swollen craniums and soft skulls, ours were healthy and were usually fair breeders. Some selection for fertility was necessary; among 177 males and 321 females placed in breeding pens, 39 and 66 respectively (21 percent) were discarded for infertility after suitable trial periods. No reason for this was apparent at autopsy.

Malformations of the heart, aorta and pulmonary arteries, such as those associated with situs inversus in man[5, 13] and those produced experimentally in rats by subjecting pregnant females to teratogenic agents [4, 14] have not been observed in our situs inversus mice. The chambers of the heart, associated vessels and lungs were always transposed together so that normal relationships were maintained. Whether the azygos vein entered the left or right superior vena cava appeared to be a matter of which embryonic vessel persisted and not of malformation of the heart.

Visceral inversion has been more extensively studied in man than in any other animal. It is rather generally agreed that the condition is not closely related to twinning although of all monozygous pairs, conjoined twins exhibit the most reversed asymmetry[5, 10, 13]. There is considerable evidence from studies of inheritance that situs inversus in man is due to a single recessive gene. However, it is so often associated with other abnormalities such as bronchiectasis, nasal polyps, and malformations of the heart, skeletal parts and other entities that it has been suggested that the inversion in some cases is a secondary rather than a primary gene effect. Torgersen[13] in a study of 168 cases concluded that, "the ratio of affected to normal persons does not accord with the supposition that a single recessive gene is always the cause of situs inversus."

Union between inferior vena cava and azygos vein has been observed in other mammals. Cooper[3] in describing this anomaly in

TABLE III. The inheritance of situs inversus viscerum

Cross	No.	Abnormal Observed		Abnormal Expected		Penetrance %
$iv+ \times iv+$	509	53	10.4%	127	25%	41.7
$iv+ \times iviv$	29	9	31.0%	14.5	50%	62.1
$iviv \times iviv$	606	429	70.8%	606	100%	70.8
normal × "reversed"	99	67	67.7%	99	100%	67.7
"reversed" × "reversed"	507	362	71.4%	507	100%	71.4

the dog, cited 30 reported cases in man as well as occurrences in the guinea pig, cat, and deer. He believed that the postcaval-azygos continuation probably represented persistence of the right embryonic supracardinal vein throughout its length. In man, cat and many other mammals, the embryonic supracardinal and subcardinal veins are important in the development of the adult inferior vena cava and azygos vein[8]. In the adult rat, on the other hand, the supra- and subcardinals play a lesser role and it is the right embryonic postcardinal vein that persists as the inferior vena cava caudal to the diaphragm (Butler[2]). The azygos vein of the rat and mouse differs from that of the cat, dog, rabbit and man in position, being on the left side and entering the left superior vena cava; description of its derivation is not included in Butler's study. Assuming that mouse and rat development are similar, the inferior vena cava-azygos junction could represent either persistence of the postcardinal-supracardinal anastomoses in the region of the diaphragm or the persistence of the embryonic postcardinal vein over its entire length[9].

The frequent association between abnormality in liver lobation, inferior vena cava bypass of the liver, and ventral position of the hepatic portal vein can not be accidental, and is indicative of a common cause. This has an important bearing on a fundamental problem in vertebrate development, the cause of normal asymmetry of unpaired organs and blood vessels. It seems hopeful that the embryonic processes important in establishing asymmetries can be defined through studies of development of these mice in which reversed asymmetry occurs as the result of gene action.

Summary

Situs inversus viscerum, inherited as a single recessive character with incomplete penetrance has been described for the second time in mice. Expression includes left-right transposition of thoracic and abdominal viscera and associated blood vessels, anomalous relationship of postcaval and azygos veins, anomalous position of the hepatic portal vein, abnormalities in spleen position and shape, and in liver and lung lobation.

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SUBSPECIFIC DIFFERENCES IN THE RESPONSES OF YOUNG DEERMICE ON AN ELEVATED MAZE

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DIFFERENTIAL rates in the morphological and behavioral maturation of several species and subspecies of *Peromyscus*[2, 5, 8, 9, 10] suggest that adult characteristics are established early in life. One behavioral characteristic which distinguishes terrestrial forms from semi-arboreal forms of *Peromyscus* is the reaction of adult mice on an elevated platform[4]. Terrestrial forms, such as *P. maniculatus bairdii, P. m. blandus,* and *P. m. nebrascensis,* tend to drop, fall, or jump from a platform more readily than the semi-arboreal forms of *P. m. gracilis, P. m. oreas,* and *P. nasutus nasutus.* Because of the differential rates of development in many of these forms, the age of the first appearance of this "falling off" characteristic in the ontogeny of terrestrial and semi-arboreal subspecies may be expected to differ. The purpose of this study was to discover the age at which this characteristic reaction to an elevated platform and any related behavior patterns first appeared in the ontogeny of terrestrial and semi-arboreal subspecies of deermice.

Methods

Animals

A total of 110 *P. m. bairdii* and *P. m. gracilis,* which had bred in the laboratory for approximately 10 years, were divided into 11 groups of 10 mice each. Five age groups at 10, 14, 18, 25, and 30 days of age from each subspecies were used for a cross-sectional analysis, i.e., each mouse was given one series of five trials at only one age. One group of 10 mice of *P. m. bairdii* was tested at the same age for a longitudinal analysis, i.e., each mouse was given repeated series of five trials as it reached each of the five ages. Birth dates of the mice were recorded within 24 hours. The subjects were selected from litters of three to six mice. Two mice from each of five litters were included in the longitudinal group and no more than two mice from any one litter were tested at the same age in the cross-sectional groups. However, as many as six mice from the same litter were sometimes used in the cross-sectional groups at different ages.

Apparatus

The apparatus was designed to provide an aversive situation on an elevated, exposed platform from which the mice could escape by falling off or by running down a passageway to an escape box. The platform consisted of an elevated Y maze, supported 2.5 ft. from the floor by a metal pole. The arms of the maze were 12 inches long and 3 inches wide. The surface of the maze was covered with a fine meshed screen. At the end of one arm, a covered screen passageway, 26 inches long, sloped at a 30° angle from the elevated maze to a 4 inch cubed escape box containing a 150 mm beaker. This passageway provided an escape from the maze and a sheltered retreat at the end in the covered box. The beaker in the box made it possible to return the mouse to the maze without handling it. In order to prevent the escape of mice that jumped from the maze, flex-o-glass was mounted on a large wooden frame in the shape of a funnel. Mice jumping or dropping from the maze were funnelled into a 150 mm

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beaker, which again enabled the mice to be returned to the maze without being handled. The mice were started from the center of the maze by raising an inverted beaker which contained the mice. The experimenter, hidden behind a cloth screen, raised the beaker by pulling an attached string leading over a pulley directly above the maze. The beaker, containing the mouse was held in place on the maze by three nails in the center. A 150 watt light and a 48 amp. electric blower were suspended three and four feet, respectively, above the maze. The blower masked incidental noises and directed a current of air directly at the maze. The light, blower, and exposed platform was considered to be an aversive situation.

Procedure

The mice were given five trials each of five minutes duration or until they left the maze. Each trial was preceded by holding the mouse 30 seconds under the beaker in the center of the maze. Mice which reached the escape box at the end of the passageway were given one minute inside before they were returned. Once a mouse went half way down the passage, its trial was completed and it was given a minute either to enter the box or to return to the maze where it was caught. Duration on the maze (length of time on maze), number of arms entered per minute, the frequency of falling or jumping from the platform, and the frequency of descending the passageway were the principal measures used. Statistical analyses were made by the use of the Mann-Whitney U test[7] which does not require the assumption of a normal distribution of the data.

Results

Since this study is primarily developmental, the presentation of the results will be organized with respect to ages tested, rather than with respect to the measures employed.

Ten days

At 10 days of age, *bairdii* appears somewhat more mature than *gracilis*. The pelage of *bairdii* is thicker, longer,

TABLE I. Mean scores and probability levels for differences between groups of *Peromyscus maniculatus gracilis, P. m. bairdii* (cross-sectional) and *P. m. bairdii* (longitudinal).

		AGES IN DAYS									
		10		14		18		25		30	
MEASURE	GROUP	Mean	P	Mean	P	Mean	P	Mean	P	Mean	P
DURATION (minutes)	Gracilis	24.2		23.4		12.7		9.4		9.5	
			.005		.005		.04		N.S.		N.S.
	Bairdii (x-sect.)	14.2		15.4		7.3		8.5		8.6	
			N.S.		N.S.		.04		.01		N.S.
	Bairdii (long.)	13.4		17.7		16.1		18.9		13.8	
ACTIVITY (arms/min.)	Gracilis	0.02		0.26		2.32		2.45		3.52	
			.005		.01		N.S.		N.S.		N.S.
	Bairdii (x-sect.)	0.37		0.69		2.27		2.99		3.68	
			N.S.		N.S.		.05		.025		N.S.
	Bairdii (long.)	0.28		0.64		1.28		1.75		2.32	
FALLING (freq. 0-5)	Gracilis	0.3		0.5		0.1		0		0.2	
			.001		.04		N.S.		N.S.		N.S.
	Bairdii (x-sect.)	3.0		1.9		0.2		0.1		0.1	
			N.S.		N.S.		N.S.		N.S.		N.S.
	Bairdii (long.)	2.8		1.4		0.6		0		0.1	
PASSAGEWAY (freq. 0-5)	Gracilis	0		0		3.3		4.3		3.9	
			N.S.		.04		N.S.		N.S.		N.S.
	Bairdii (x-sect.)	0		1.3		4.0		4.3		4.2	
			N.S.		N.S.		.02		.001		.04
	Bairdii (long.)	0		0.9		1.4		1.3		2.6	

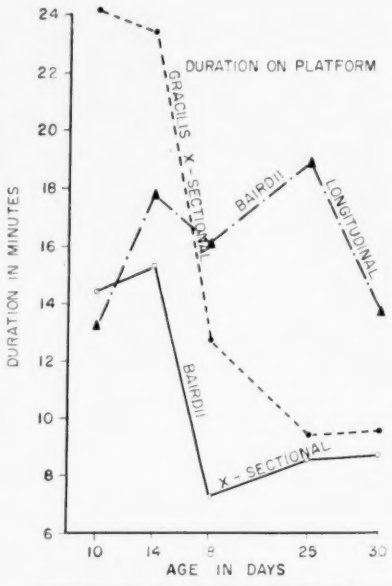
and sufficient to cover the ventral surface. Although the eyes of both subspecies are usually closed, the eye slits are deeply grooved in *bairdii*, and an occasional individual may have its eyes open. In general body proportions, *gracilis* have a more infantile appearance with a relatively larger head. The posture of the two subspecies is noticeably different: *bairdii* usually have the limbs under the body and are capable of sitting, while *gracilis* still extend their limbs laterally, particularly the hind limbs. Locomotion by *bairdii* may be characterized as a walk, while in *gracilis* it is still a crawl.

The behavior of the two subspecies on the elevated maze was significantly different in three of the four measures (Table 1). *Bairdii* spent significantly less time on the maze (Figure 2), were more active, and fell off more frequently than *gracilis*. Neither subspecies used the passageway to escape from the maze at this age. Since most of the measures are related, the principal difference between the two subspecies can be attributed to the greater activity of *bairdii*. In their movements about the maze, their feet would frequently slip off the side and the mouse would fall. *Gracilis* movements were primarily limited to pivoting about in the center of the maze where they were released. *Bairdii* were frequently observed grooming themselves with their forepaws in a sitting position, while *gracilis* rarely groomed at this age. Both subspecies squeaked frequently.

The longitudinal *bairdii* had equivalent experience to the cross-sectional *bairdii* at this age and did not differ significantly from them.

Fourteen days

The degree of physical maturation in the two subspecies is conspicuously different at this age. The young *bairdii* have their eyes open, they are well-furred, their limbs are drawn under them and can easily support their bodies. They still have the blunt noses of the infant and their ears are relatively small in relation to the size of their head. Young *gracilis* at this age rarely, if ever, have their eyes open, the skin is still



BEHAVIOR OF THREE GROUPS

Figure 2

Mean number of minutes spent on an elevated platform (duration) during five trials at five age levels by three groups of *Peromyscus maniculatus*.

visible through the ventral pelage, and their limbs are characteristically extended laterally, although they can be drawn under the body to support it as it moves.

Behaviorally, the two subspecies were significantly different in all four of the measures (Table I). *Bairdii* were very active, running about on the maze, sometimes falling off in the process, and almost as frequently finding the passageway leading from the maze. Once they located the passageway, they continued to escape from the maze in this way. A few individuals froze or remained motionless on the maze platform. They rarely squeaked. On the other hand, *gracilis* squeaked frequently and moved about very little. When they did move, it was usually to the end of one of the maze arms, where they remained. If they went on the arm with the passageway, they frequently descended part way and

then returned. They never went entirely down the passage. At the end of the arms, they were often actively engaged in turning about and looking over the edges. They did not usually return to the center and explore the other arms of the maze.

The previous experience of the longitudinal *bairdii* on the maze did not affect their behavior sufficiently to make them significantly different from the cross-sectional *bairdii*.

Eighteen days

At 18 days, the differences in the physical maturation of the two subspecies are no longer apparent; however, subspecific differences in pelage color, ear size, and tail length are conspicuous. Both subspecies have their eyes open, their noses are pointed, and their bodies have grown more in proportion to the size of their heads.

The behavioral development of the two subspecies paralleled their physical development to a certain extent. Both subspecies became much more active on the maze platform, but *bairdii* spent significantly less time on the maze before descending the passageway than did *gracilis* (Table I). It was typical for *gracilis* to descend part way down the passageway and then return to the platform. *Bairdii* usually went all the way down to the box at the end of the passage and once this retreat was located, they often ran directly to it when released. *Gracilis* still squeaked frequently in this situation.

While the two subspecies were similar in their performance on the maze, with the exception of duration, the longitudinal *bairdii* were significantly different from the cross-sectional *bairdii* in duration, activity, and frequency of descending the passageway (Table I). Falling was rare in both groups at later ages. The longer duration, reduced activity, and infrequent descent of the longitudinal group was a reflection of their tendency to walk out to the end of the arm, groom themselves, and then merely sit, almost as if they were asleep. This immobility on the part of the longitudinal group did not resemble the "fear-induced" freezing of some of the cross-sectional mice.

Twenty-five days

The tendency for both subspecies to behave similarly in the test situation continued and at 25 days there were no longer any significant differences in any of our measures. In contrast, the differences between the longitudinal and cross-sectional *bairdii* groups became highly significant (Table I). The longitudinal *bairdii* were less active and remained on the platform longer than the cross-sectional *bairdii*. The characteristic response of the longitudinal mice was to go out to the end of an arm and remain motionless throughout the five minute trials.

Thirty days

The behavioral trend of *bairdii* and *gracilis* to respond to the test situation in the same way was continued at 30 days of age; there were no significant differences in their performances. Although the longitudinal *bairdii* still spent more time on the platform, were less active, and descended the passageway less frequently than the cross-sectional *bairdii*, the differences were significant only with respect to frequency of escaping by the passageway (Table I).

Discussion

Although the behavioral differences between the two subspecies parallels their physical maturation at the age levels examined, there are some differences which cannot readily be attributed to maturation alone. Among the four measures used, the characteristic of falling off the elevated maze was significantly more frequent in *bairdii* than in *gracilis*, particularly at 10 and 14 days of age. This agrees with Horner's study of adult mice[4]. The fact that this characteristic disappeared in our older animals is probably due to a difference in apparatus. Unlike Horner's, our apparatus offered the mice an opportunity to escape by the passageway. That falling off is not entirely related to activity at an early age is indicated by its almost complete absence in *gracilis* at any age, even after they become quite active. This difference in falling off an elevated substratum is apparently a subspecific characteristic, which differentiates the two subspecies at about 10 days of age.

If we assume that this subspecific difference is genetically determined, the problem of genic

action remains. Usually innate behavioral responses are considered the result of some complex physiological reaction or structural difference in the nervous system[3, 6]. Another possible interpretation is that the differential rates of development in locomotion and the clinging response establish rigid patterns of behavior which may endure throughout the life of the organism. According to this hypothesis, *bairdii* become physically capable of locomotion prior to the development of a strong clinging response, while *gracilis* develop their locomotor capacity and clinging tendency simultaneously. Differential rates in morphological development are well established[1]. Once falling or the opposite response, clinging, is established early in life, it tends to persist throughout the life of the mouse.

This characteristic is certainly only one of many other characteristics which might exhibit differential maturation rates and, when interacting with the genetic predisposition of the various species and subspecies of *Peromyscus*, might well account for the differences in behavior observed throughout this genus.

The difference in maze performance at later ages between the longitudinal and cross-sectional groups of *bairdii* can be attributed to habituation to the aversive situation on the lighted, windy, exposed platform. Another possibility is that this difference is due to the failure to provide an adequate reinforcement in the escape box for the longitudinal mice. Since none of the mice went down the passageway at 10 days and only six went down at 14 days for a total of nine times out of a possible 50 times, their previous experience with the escape box was too limited to be seriously considered either as positive or negative reinforcement. Habituation seems more likely to account for the data. The 50 minutes of experience on the platform at 10 to 14 days of age was apparently sufficient to habituate the mice to the situation, so at 18 days of age the response was significantly different from the cross-sectional mice of the same age after the first trial. The fact that the 18-day old cross-sectional mice failed to show any indication of habituation by their fifth trial suggests that the longitudinal mice may have habituated to this aversive situation prior to the age their sensory-motor capacities were sufficiently mature to enable them to respond like the cross-sectional 18 day mice. On the other hand, the cross-sectional mice might have matured to the extent that the characteristic behavior pattern was an attempt to avoid the situation and habituation may have been prevented.

Summary

Two subspecies of *Peromyscus maniculatus* were studied in cross-sectional groups at five ages, with 10 individuals representing each age class. One longitudinal group of one subspecies was tested at the same five ages throughout their early development. The mice were placed in an aversive situation created by an elevated Y maze which was provided with one escape route to a sheltered box. Each mouse in the cross-sectional groups was given five trials of five minutes duration or until they escaped from the maze. The mice in the longitudinal group were given the same procedure at each age, giving them a total of 25 trials upon completion of their tests. Latency to escape, activity, and method of escape were used as measures of their behavior.

The subspecies behaved differently at 10 and 14 days of age in most measures, but responded alike at 18, 25, and 30 days of age, except for latency at 18 days.

The longitudinal and cross-sectional groups of one subspecies behaved similarly at 10 and 14 days of age, but responded differently to the situation in most measures at 18 days and later.

Differential rates of maturation in activity and the clinging response may serve as an explanation of the subspecific differences found early in development.

The later differences between the cross-sectional and longitudinal groups are the result of prior experiences on the platform which apparently habituated them to the aversive situation.

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SELECTION AND INBREEDING FOR LONGEVITY OF A LETHAL TYPE

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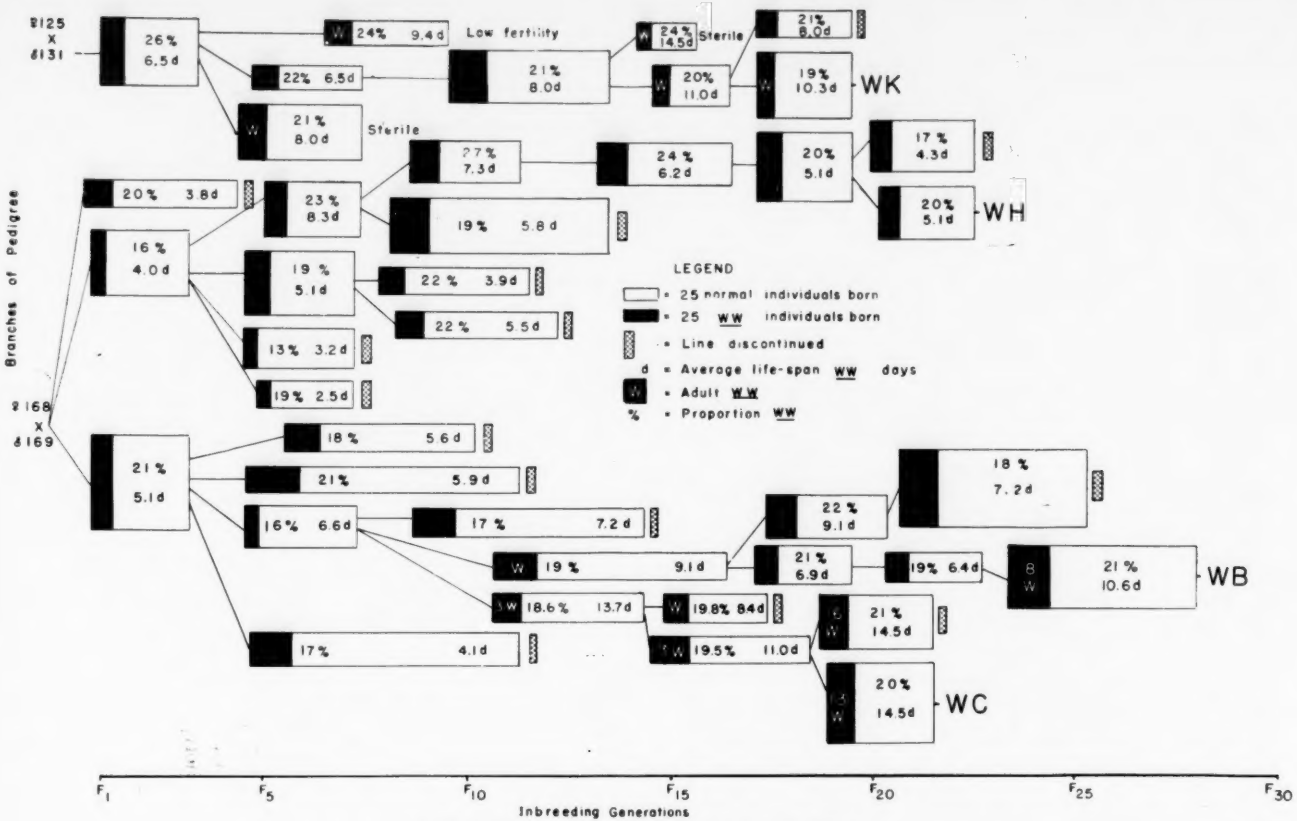
PROGRESS in analysis of the action of particular mammalian genes is frequently dependent upon the results of prolonged genetic manipulation leading to the development of animal strains as nearly as possible isogenic except for the genes under investigation. Quantitative evaluation of differences between genotypes depends upon existence of a common genetic background. Studies of time and place of gene action are greatly facilitated by intergenotypic transplantation of tissues, possible only if the tissues are immunogenetically compatible. One extensively used method for producing isogenic stocks with and without a particular gene is repeated backcrossing of individuals, carrying one allele, to a pre-existing inbred strain which lacks it. This process in eight generations will theoretically produce more than 99 percent isogenicity at other loci. It is difficult, however, to determine in advance the effects of a given inbred background upon penetrance or expressivity of a particular gene. This is important in studies involving a lethal genotype, since a genetic background favoring live-birth of severely affected individuals greatly simplifies analysis of gene-action. Hence it may sometimes be easier to develop a new inbred strain by brother-sister matings with forced heterozygosis at one locus than to use the backcross method.

These considerations have been of great importance in planning the development of stocks of mice segregating for genes of the *W*-series. Animals of six of the genotypes (WW^J, WW^v, W^JW^J, W^JW^v, W^vW^v) are black-eyed white, anemic, and sterile, and two of these (WW and W^JW^J) are usually lethal. Repeated backcrossing of mice carrying W to C57BL/6 failed to produce a useful stock, since in matings between Ww individuals only 4 percent, rather than the expected 25 percent, of WW individuals was born alive, and the average survival was two days. Following the appearance in 1949 of two long-surviving WW animals (15 and 19 days) in a genetically heterogeneous colony, a breeding program of heterozygous Ww matings was initiated at the Jackson Laboratory. This involved a combination of mild selection at the individual level, severe inter-group selection, and continued brother-sister inbreeding. The program has produced four inbred mouse strains especially favorable for studies of action of the *W*-series genes. The basis of selection was the behavior of WW individuals. Since these are completely sterile, the animals selected as parents to continue the line were their Ww siblings. This paper is presented to record the methods used in this selection-inbreeding program, and their success as indicated by the history of each branch of the colony pedigree. A brief description is also given of presently known characteristics of the four new inbred strains which have resulted from this breeding program.

Materials and Methods

The original stock used to start the brother-sister mating was derived from crosses involving Ww animals, received from Dr. S. J. Holman and Dr. Salomé Waelsch, as well as a few C57BL/6 animals from the Jackson Laboratory Pedigreed Expansion stock. At the beginning

* Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This work has been aided by grants from the Atomic Energy Commission and the American Cancer Society. In early stages of this program, large bodies of data were collected by Louise M. Murray, Eleanor M. Small, Lois Jean Smith, and Willys K. Silvers. The authors wish to express their indebtedness to these faithful helpers.



FREQUENCY AND SURVIVAL OF *WW* MICE

Figure 3

Pedigree chart showing frequency and survival of *WW* anemic mice during selection and inbreeding program.

of the experiment, all animals were maintained on Purina Fox Chow, after 1953 on Purina Laboratory Chow. Single pair matings were used throughout, with young raised in the home pen. From each *Ww* brother-sister pair, five litters were obtained. The number of normal (*ww* and *Ww*) and anemic (*WW*) offspring born was recorded for each litter, and survival of *WW* offspring was determined by observation six days per week during the period from 1949 to 1955, thereafter three days per week. One new breeding pair was made up from each existing mating; if a mating was especially successful, the new mating pairs made up were increased to two. After each four or five generations of expansion, the breeding records of all matings were placed on a pedigree chart, and behavior of different portions of the pedigree compared. Total offspring per mating, percent *WW*, average life-span of *WW* individuals, and longest life-span of *WW* individuals, determined for each portion of the pedigree, formed the basis for stringent intergroup selection. Unpromising groups were discontinued.

Results

A summary of the changing pattern of frequency and survival of *WW* individuals during the selection-inbreeding period is given in chart form in Figure 3. Each block on the chart represents a portion of the pedigree which was considered as a unit in intergroup selection. The size of the block depends upon the total number of offspring born to the matings in that section of the pedigree (combining together all the sets of five litters produced by the females within the group). The white portion of the rectangle represents the number of normal (*Ww* and *ww*) offspring, the black the number of *WW* anemic offspring. The percentage value given in each rectangle (26 percent in the top left block) is the observed percent of *WW* individuals among live-born offspring. The number (6.5d in the top left block) is the mean days survival of these *WW* individuals, with any surviving to adulthood calculated as living 40

TABLE I. Breeding behavior of females born in 1956 in colonies of four inbred strains maintained heterozygous for *W/w*. (All matings were *W/w* × *W/w*)

Inbred strain	F generations included	No. of ♀♀	Age at 1st litter mean ± S.E. (in days)	Number of offspring per litter													
				1st litter			2nd litter			3rd litter			4th litter			5th litter		
				No. litts.	Mean born	% *WW*	No. litts.	Mean born	% *WW*	No. litts.	Mean born	% *WW*	No. litts.	Mean born	% *WW*	No. litts.	Mean born	% *WW*
WB	24-27	38	75.3 ± 1.2	38	4.9	18	38	5.9	15	38	5.2	23	37	4.9	24	32	5.1	18
WC	19-22	43	91.9 ± 1.5	43	5.7	25	43	5.1	18	43	5.4	23	40	4.8	25	35	4.5	19
WH	22-25	22	85.8 ± 3.1	22	4.0	15	22	4.9	20	22	4.5	14	22	4.4	16	19	4.1	17
WK	18-21	22	85.5 ± 3.7	22	5.0	20	22	4.9	24	21	5.1	20	20	5.1	20	16	5.8	15

days. In preparing the chart, care was taken to have each block end at the most advanced inbreeding generation in that particular population at the time of analysis. However, the rectangular shape chosen to make evident the relative sizes of different populations prevented accurate depiction of the F-generation beginning a new population unit. If two populations descended from a single mating in a former pedigree unit (common ancestor of WB and WC in F_8, for example), the two lines connecting the old to the new blocks were given a common base-point.

This selection-inbreeding program has led to the establishment of four inbred strains, each descended from at least 20 successive generations of mating between full-sib *Ww* individuals. Six of the 11 groups carried as far as F_5 were discontinued at or before F_{12} (Figure 1). The discarded lines showed *WW* percentages ranging from 13 percent to 21 percent and mean survival of *WW* individuals ranging from 2.5 to 5.9 days. Two other lines which showed promising *WW* survival (21 percent and 24 percent *WW* born, mean survival, 8.0 and 9.4 days) were lost, due to low fertility, at F_7 and F_{10}. In each of the two groups which were lost, one *WW* individual survived to adulthood.

Three lines, which between F_5 and F_8 had 16 percent to 23 percent live-born *WW* individuals, with mean days survival ranging from 6.5 to 8.3 days, were continued, and are the ancestors of the present inbred strains.

Two inbred strains in which selection for frequency and longevity of *WW* was successful, WB and WC, are descended from a single pair of animals in F_8. These parents came from a pedigree group which at F_{5-8} did not appear especially promising (16 percent *WW*, mean survival 6.6 days). The improvement of *WW* longevity in the ancestors of WC was sudden and dramatic: between F_{12} and F_{13}, three of 49 *WW* individuals survived to adulthood, and the mean survival of all *WW* individuals was 13.7 days. This high performance still continues, with better than 10 percent of *WW* mice surviving to adulthood. Text Figure 3 shows two equally good portions of the WC line separated at F_{19}. Since these were so much alike, decision as to which should be continued was strictly arbitrary. Improvement in *WW* survival among the ancestors of the WB strain came later and with less certainty. The most recent portion of the WB pedigree (F_{24-28}) shows eight adults among 133 *WW* individuals born, and an average survival of 10.6 days.

The third inbred strain with improved *WW* survival, WK, has a completely separate pedigree, including side branches with high *WW* survival which were lost due to infertility. The present WK strain shows 19 percent *WW* with a mean survival of 10.3 days.

The ancestors of the fourth line, WH, showed some improvement in *WW* survival until F_7, but declined in later generations, although subjected to the same regimen of selection as were ancestors of the other lines.

It may be worthwhile to give some consideration to other characteristics fixed in these four inbred strains. Characters which have been studied to date are breeding behavior within the strains, breeding behavior and survival of WW^v severe anemic animals in the F_1 hybrids

TABLE II. Data on survival of severely anemic (WW^v) offspring born 1956-1957 in each of four types of F_1 hybrid matings

F_1 hybrid	Number breeding females	Offspring born: Total number	WW^v: No.	WW^v: Percent	WW^v survival weeks: <2	2-3	3-4	4-5	>5 (adults): No.	>5 (adults): Percent
WB-*Ww* × C57BL-W^v	77	1928	462	24.0	91	96	78	70	127	27.5
WC-*Ww* × C57BL-W^v	75	1844	472	25.6	101	71	98	80	119	25.2
WH-*Ww* × C57BL-W^v	20	512	130	25.4	22	30	24	14	40	30.8
WK-*Ww* × C57BL-W^v	55	1422	349	25.8	90	51	61	55	90	25.8

of each of these strains with C57BL/6, and certain hematological characteristics of newborn and young adult mice.

Analysis of breeding records of the four strains shows them to differ somewhat in performance. In 1956, when each of the strains had achieved a high degree of homozygosity, the mean number of live-born offspring, and the proportion among them of *WW* individuals, was lower through most of the litter seriation (1st, 3rd, 4th and 5th litters) in the WH strain than in any other (Table I).

Females of the four strains also differ in mean age at first litter (Table I). Females of the WB strain produce their first litters significantly earlier (mean, 75.3 ± 1.2 days) than do females of the WC strain (mean, 91.9 ± 1.5 days). Females of the other two strains give intermediate values.

Matings between *Ww* individuals from each of these inbred strains and *W*^{s}*w* individuals isogenic with C57BL/6 have proven reliable sources of severely anemic *WW*^{s} adults. During 1956-57, in each type of F_1 hybrid mating, the full expected ratio of *WW*^{s} offspring was born alive (Table II). In all types, there was considerable loss of anemic offspring before weaning age, but the proportion of surviving adults among *WW*^{s} anemic individuals born was very similar for all four hybrid crosses.

Determinations of the mean erythrocyte levels of normal (*Ww* and *ww*) and anemic (*WW*) newborn mice in each of the four inbred strains demonstrate significant strain differences in the mean erythrocyte count of normal newborns (Table III). In each of the four strains, the mean erythrocyte count in *WW* newborns is close to 30 percent of that characteristic of newborn normals. Typical erythrocyte numbers, hematocrit percentages, and mean cell volumes of young adults (three months) have been determined for each of the four inbred strains (Table IV). Values for *Ww* and *ww* adults are presented separately. In each of the inbred strains, the number of erythrocytes/mm^3 for *ww* and *Ww* normal adults is approximately 10-11 $\times 10^6$, with slightly higher mean values for *Ww* than for *ww* individuals in three of the four inbred strains. All have approximately the same mean cell volume (means ranging from 43.2 to 46.3 μ^3). In the WC inbred strain, *WW* surviving adults have occurred with sufficient frequency to allow determination of characteristic adult blood values for animals of this genotype also. Their mean number of erythrocytes per unit blood volume (4.64×10^6 RBC/mm^3) is less than half that observed in normal adults, but represents a considerable increase both in total erythron and in erythrocytes per unit blood volume over the level observed in the much smaller newborn animals of the *WW* genotype (mean value, 1.68×10^6 RBC/mm^3). The erythrocytes of adult *WW* anemics are much larger than those of comparable normals.

Discussion

The pattern of *WW* survival in the pedigree shown in Figure 3 indicates that genetic factors capable of altering *WW* longevity were present in the original heterogeneous population, or have arisen during inbreeding, and that some or all of them have been fixed in one or another of the inbred populations. Environmental factors, such as the dietary change previously mentioned, may have altered

TABLE III. Mean erythrocyte numbers of normal (*Ww* and *ww*) and severely anemic (*WW*) newborn mice (0-1 day) in each of four inbred strains maintained heterozygous for *Ww*

	RBC/mm^3 $\times 10^{-6}$				
	Normal (−*w*)		Anemic (*WW*)		
Inbred strain	No. mice	mean ± S.E.	No. mice	mean ± S.E.	Ratio, −*w*/*WW*
WB	29	5.58 ± .16	27	1.53 ± .07	.27
WC	39	5.24 ± .12	26	1.68 ± .09	.32
WH	21	6.55 ± .24	20	1.83 ± .09	.28
WK	15	6.04 ± .17	15	1.81 ± .09	.30

expression of some genes. The basis of selection used was the average percent live-birth and average days survival of *WW* offspring in a large group of related matings. The individuals whose characteristics form the basis for selection are themselves sterile.

For two reasons the selection used was milder at the individual than at the related-group level. The first reason is that the characteristics being studied, live-birth and survival of a defective type of individual, may be strongly affected by non-genetic variables, whose effects would tend to cancel out in a large group of related matings. The second reason for mild individual selection is that favorable modifying genes present in *WW* individuals are not necessarily also fixed in their *Ww* siblings, which become the parents of the next generation. If, however, evidence is found of the presence of such favorable modifying genes in most of the anemic *WW* individuals from a group of related matings, it becomes much more probable that these same genes are also fixed in the normal *Ww* and *ww* mice within this group, and thus will be carried to later generations through the *Ww* siblings used as parents.

Although the selection program had little effect on the proportion of live-born *WW* individuals in all groups, the survival potentialities of these postnatal *WW* individuals differ in the four inbred strains which have been developed. In one strain, WC, *WW* adults are available fairly regularly, and the mean lifespan of *WW*s is 14.5 days. In another strain, WH, the mean lifespan of *WW* individuals is only 5.1 days. Survival of *WW* individuals in the WB and WK strains is intermediate between these extremes. It appears that in the course of inbreeding, different genes affecting the survival potential of *WW* mice have become fixed in these four inbred strains.

Comparison of other characteristics of animals from these strains might disclose genetically determined differences showing some correlation with *WW* survival potentiality. One might expect such differences to be related to the hematological picture. However, determinations of the erythrocyte levels in normal and anemic newborn mice of each inbred strain failed to yield any such correlation, the strain (WH) with the highest RBC/mm^3 in *WW* individuals having the lowest survival potentiality. Neither were there differences in erythrocyte number or size among adults of the different strains which appeared related to *WW* survival potentiality.

Since most of the *WW* individuals die during the suckling period, differences in breeding behavior or in maternal care might have some significance. In this case, there is some possible relationship between the relatively poor breeding performance of the WH inbred strain (smaller litters with fewer *WW* individuals than any other group) and the short lifespan of liveborn *WW*s produced in this strain. Aside from this one possibility, there were no differences in age at first litter or in litter size with apparent relationship to longevity of *WW* offspring.

In addition to the observation of survival of *WW* offspring born in each of these four inbred strains, observations have also been made of the survival potentialities of *WW*^{v} severely anemic offspring in each of the four F_1 hybrids between these four inbred strains and C57BL (Table II). In these outcrosses, all *WW*^{v} individuals appear to be born alive, and approximately one-fourth to one-third survive to adulthood. The proportion of *WW*^{v} individuals surviving is approximately the same in each of the hybrid types, ranging from 25 percent in WC $\times$ C57BL to 31 percent in WH $\times$ C57BL. The modifying genes which, when present as homozygotes in the WC inbred strain, lead to especially long survival of *WW* individuals, have no such favorable effect on anemic survival when present as heterozygotes in the WC $\times$ C57BL outcross.

Thus studies of hereditary characteristics established in these four inbred strains, developed in a program combining brother-sister inbreeding with a program of selection between groups of related matings, have indicated the existence of genetic factors modifying survival potentialities of animals of the severely anemic *WW* genotype, but have so far given very little evidence as to the physiological or anatomical basis for the observed differences in survival.

Whatever may be the mechanism of action of the favorable modifying genes, it is clear that the selection has been successful. In all

TABLE IV. Erythrocyte values for young adults of each of the *W* genotypes occurring regularly in the four inbred strains maintained heterozygous for *Ww*

Inbred strain	*W* genotype	No. of mice	RBC/mm^3 $\times 10^{-6}$ Mean $\pm$ S.E.	Hematocrit % Mean $\pm$ S.E.	MCV, mu^3 Mean $\pm$ S.E.
WB	*ww*	19	10.31 $\pm$ .18	46.7 $\pm$ 0.4	45.5 $\pm$ 0.7
	Ww	20	11.11 $\pm$ .13	48.9 $\pm$ 0.4	44.1 $\pm$ 0.4
WC	*ww*	20	10.35 $\pm$ .15	47.7 $\pm$ 0.2	46.3 $\pm$ 0.7
	Ww	20	10.84 $\pm$ .20	47.4 $\pm$ 0.4	44.0 $\pm$ 0.7
	WW	6	4.64 $\pm$ .22	32.3 $\pm$ 1.1	69.9 $\pm$ 2.3
WH	*ww*	18	10.25 $\pm$ .19	46.0 $\pm$ 0.6	45.2 $\pm$ 1.2
	Ww	21	10.18 $\pm$ .16	45.3 $\pm$ 0.5	44.7 $\pm$ 0.7
WK	*ww*	18	10.95 $\pm$ .21	48.0 $\pm$ 0.5	44.0 $\pm$ 0.7
	Ww	16	11.11 $\pm$ .25	49.3 $\pm$ 0.4	43.2 $\pm$ 0.8

four inbred strains, there are live postnatal *WW* anemic mice with which to work. In one strain there are some adult *WW* individuals for study. Hybrid crosses between these four inbred strains and C57BL produce a reliable supply of WW^v severely anemic animals for experimentation. In each of these inbred strains and F_1 hybrids, transplants of tissue may be made between histocompatible, genetically comparable normal and sterile anemic mice[1, 2]. Four new inbred strains of mice especially useful for research in the *W*-anemias, have been produced.

Summary

Four new inbred strains of mice especially favorable for studies of the *W*-anemias have been developed by a program of selection and inbreeding. The survival potentialities of postnatal *WW* anemic animals differ in each of these strains. Differences in erythrocyte number and size among adults of the new strains show no correlation with *WW* survival potentiality. The pattern of survival of anemic individuals suggests that factors capable of altering *WW* longevity have been fixed in one or another of the inbred populations.

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RATES OF SUBLINE DIVERGENCE IN HIGHLY INBRED STRAINS OF MICE

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OUR knowledge of mutation rate is almost entirely confined to mutations of sizable effect. The usefulness of such measures of mutation is rather limited for the utilizable variation in the vast majority of traits of evolutionary and economic importance is thought to arise from the accumulations of mutations of small effect, the polygenic mutations. From this standpoint, it would certainly be desirable to obtain information on the rates of polygenic mutation affecting many different kinds of traits and from many different species.

Up to now, work done by investigators interested in polygenic mutation has been centered on the problem of inducing such mutations by irradiation as a means of augmenting the effectiveness of artificial selection. Clayton and Robertson[2] have briefly reviewed the literature of this work. Investigations of spontaneous polygenic mutation have been limited mainly to studies on chaeta number in *Drosophila*[2,4].

Polygenic mutations have otherwise received concern recently in regard to their possible effects in highly inbred strains of mice. Emphasis placed on experiments which depend upon the stability of specific traits of highly inbred mice, as for example the strains of mice so plentifully used in cancer studies, has brought attention to the problem of drift due to random fixation of polygenic mutations. The validity of making comparisons of treatments between different colonies or between different generations of a strain may be questioned on genetic grounds if the rate of such drifting is sufficiently great.

Deol *et al.*[3] and Carpenter *et al.*[1] have analyzed a number of sublines of the C57 group of strains of mice in respect to several skeletal variants, or "phenodeviant" traits. Genetical changes differentiating the sublines occurred fairly frequently, indicating that inbred strains might not be the stable entities that they so often are held to be. This type of data however does not readily permit the placing of differentiation in terms comparable with other types of data such as the normally distributed quantitative traits used in the papers cited above[2,4] and to be presented below.

One difficulty which confronts us in computing polygenic mutation rate is our inability to observe effects of the newly arising mutations at individual loci. As in quantitative genetics in general where we cannot deal directly with individual loci, we are forced to work in terms of variance. We can then use for our measure the rate at which the newly arising genetic variance of a particular quantitative trait appears in each generation. In so doing, however, we must be content with the necessity of having the mutation rates of all the loci affecting the trait and the sizes of their effects confounded in our estimate.

The following analysis has been based on the increments in genetic variance arising between sublines of two highly inbred strains of mice. The method is relatively sensitive and convenient for detecting such increments.

Materials and Methods

Two highly inbred strains of mice were available for this study, each of

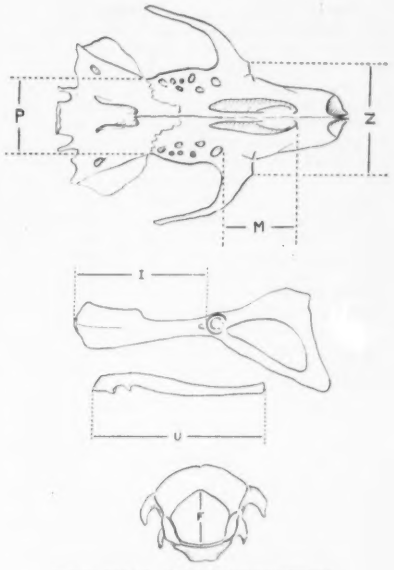
*The data for this study were gathered and partially analyzed at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, under contract No. AT (30-1)-1272 with the Atomic Energy Commission and research grant RG-4867 from the National Institutes of Health. The analysis was completed at the Mammalian Genetics Center, Department of Zoology, University of Kansas, Lawrence, Kansas and was supported by research grant RG-5658 from the National Institutes of Health. I am grateful for the help with the Appendix so generously given by Professor E. R. Dempster, and for the technical assistance of Mrs. F. Leland and Mr. J. Lambert. Present address: Division of Research Services, National Institutes of Health, Bethesda, Maryland.

which had been divided into sublines at a prior time in their inbreeding history. The C57BL/6 strain had been divided into the Jax and p sublines after more than 30 generations of full-sib matings. The BALB/cAn strain had been divided into the X and Y sublines after 78 generations of full-sib matings. There was an average of 46.5 and 9.0 generations of separation for the lines of the C57BL/6 and BALB/cAn strains, respectively.

Skeletons of 90-day old female mice, prepared by the papain-digestion technique, formed the basis of this analysis. Data were collected according to the following hierarchy: two females from each litter; one, two or three litters from each mother; 17 and 22 mothers from the sublines of the C57BL/6 strain and nine mothers from each subline of the BALB/cAn strain. The skeletons were collected from litters of mated pairs of varying numbers of generations of subline separation (from six to 12 in the BALB/cAn strain and from 41 to 50 in the C57BL/6 strain). We have used the average number of generations of separation in calculations below.

These mice were all raised in the same colony under similar environmental conditions. Their skeletons were collected during the same period so that seasonal effects would not be confounded with subline differences. Effects of possible subclinical infections associated with sublines were not eliminated by the procedures used in these analyses.

The six skeletal measurements chosen for analyzing subline divergence are shown in Figure 4. Measurements M, Z, and P are the skull dimensions in ventral aspect shown, F is the height of the foramen magnum, U is the length of the ulna, and I is the length of the ilium. Measurements were made with a "travelling" microscope with an accuracy of .01 mm.



SKELETAL MEASUREMENTS
Figure 4
Camera-lucida drawings indicating the measurements of the skull, ulna and ilium used in the analysis.

Analysis

The measurements were analyzed by an analysis of variance of the heirarchical type. The

TABLE I. Mean squares computed from analysis of variance of heirarchial type

Source of variance	Degrees of freedom	Measurement M	Z	P	F	U	I
C57BL/6							
Between sublines	1	.2133**	.4206**	.0334	.0002	3.4133**	2.7395**
Mothers within subline	37	.0145	.0377	.0145**	.0170	.0751**	.1373**
Litters within mother	15	.0103	.0282	.0068	.0165	.0508	.0765
Individuals within litter	54	.0092	.0244	.0075	.0126	.0299	.0492
BALB/cAn							
Between sublines	1	.0406	.2644	.0430**	.0725**	.9840**	1.2420**
Mothers within subline	16	.0117	.1065**	.0087	.0085	.0914**	.1631
Litters within mother	16	.0147	.0401	.0051	.0050	.0435	.1171*
Individuals within litter	34	.0076	.0341	.0077	.0094	.0320	.0471

*Significant at 5 percent level.
**Significant at 1 percent level.

mean squares are given in Table I. The subline components of variance, in which we have particular interest, were extracted from the mean squares (Table II).

These estimates do not allow us to make comparisons either among themselves or with estimates from other sources. It is necessary to express them in some common unit and also in terms of the amount of change per generation. The total genetic variance found in a population of unselected, highly inbred strains (twice that in a panmictic population according to Wright[5]) would be an acceptable standard since it would place the increments in terms of the total attainable without selection. Such standards are far from ideal, for estimates of genetic variance vary from population to population and in the same population lineage through time. Nevertheless we shall make use of those estimates of genetic variance available to us.

For this purpose, we have made estimates from data coming from two independent sources: (1) a group of 10 highly inbred strains (these are listed at bottom of Table III) and (2) a group of 100 inbred lines. The latter group had originated from a double-hybrid cross (involving strains: Small (McArthur), Large (Goodale), DBA/1, and 129) and was subsequently maintained by crosses of minimum inbreeding for three generations prior to being separated into the 100 lines. These lines were then maintained by full-sib matings for three generations. (Since these lines of mice were originated for another investigation, we shall not describe them in greater detail here.)

From these two groups of data we obtained for each variable two independent estimates of the genetic variance that would be expected among highly inbred strains. Of these two estimates of variance, we expect the first to be lower than the second, for some of the 10 strains used originated from common sources and thereby the inter-strain (genetic) variance would tend to be reduced. On the other hand, the strains used in the double-hybrid cross were of extreme types, the Large and Small strains having been selected for large and small size, thereby the estimates of genetic variance coming from them would tend to be augmented. This expectation was borne out for all but the variances of P and F measurements, the estimates of which did not appreciably differ (see Table III). The average of the two sources of estimate have been used in expressing the divergence estimates in terms of genetic variance.

Results and Discussion

Rates of genetic divergence per generation have been computed as a proportion of the genetic variance expected in a population of highly inbred strains. These are presented in Table IV for both strains and for the six measurements, arranged in the anterio-posterior order as they appear in the mouse.

Subline divergence of the magnitude found here would present a rather serious problem to anyone interested in maintaining "standardized" material with which to work. As an example, suppose we were to compare the means of skeletal dimensions for two groups, each group coming from a separate subline and consisting of 10 mice each. We might then inquire as to how many generations might elapse before the differences between the two groups would become significant. Estimates of the number of generations elapsing before the differences would be considered significant at the 5 percent level are given in Table V for the traits used in this study. These estimates were derived from the estimates of genetic divergence in Table IV and from the estimates of error variance obtained as an average of those coming from the highly-inbred strains, the double-hybrid lines and the BALB-cAn and C57BL/6 strains, all of which have been described above.

Estimates in Table V refer to differences of two sublines originating contemporaneously

TABLE II. Estimates of between-subline components of variance

	Measurement					
Strain	M	Z	P	F	U	I
C57BL/6	.0037	.0071	.0003	.0000*	.0616	.0478
BALB/cAn	.0008	.0041	.0010	.0019	.0258	.0315

*Between-sublines mean square smaller than within-sublines mean square.

TABLE III. Estimates of genetic variance expected between highly inbred strains

	Measurement					
Source	M	Z	P	F	U	I
Double-hybrid data	.066	.212	.043	.044	.330	.608
Inbred-strains data*	.037	.068	.045	.059	.119	.309
Average	.052	.140	.044	.052	.224	.458

*A/Jax BALB/cAn C3H/Jax DBA/Wa SWR AKR CBA C57BL/6Jax LP 129

and separated for the stated number of generations. If we were to compare a single line at two points in time, the numbers of generations as given should be doubled. It is apparent from these estimates that genetic drift involving polygenic mutations may be an important source of bias if left unheeded in experimental design.

The rates of increase of genetic variance of chaeta number in *Drosophila* have been estimated by Clayton and Robertson[2] and by several other sources presented by Mather[4]. These estimates were expressed as proportions of the genetic variance of chaeta number estimated to be extant in wild panmictic populations. Their estimates all approximated .001 per generation. Admittedly, there is not much value in comparing statistics of this sort between such diverse organisms as the fruit fly and the mouse. However, with the present state of knowledge it may be interesting to see the diversity of such values in a wide range of organisms.

As they stand, our values can be compared with those of *Drosophila*. It is shown in the appendix to this paper that what we have termed "rate of subline divergence" is equivalent to the rate of accumulation of genetic variance in a panmictic population. Our estimates, although ranging from 0 to 0.013, are generally higher than the estimate given for chaeta number. In fact the one value for ulna length of the BALB/cAn sublines is over 10 times greater. Whether this difference, if real, is due to a higher mutation rate, greater effects of individual mutants or a greater number of effective loci, we cannot say.

There are several questions which this analysis evokes but cannot answer. The consistently higher values for the BALBc/An strain in Table IV suggest that the sublines of this strain are more prone to differentiate than are those of the C57BL/6 strain. However this may very well be a spurious result due, say, to a rare mutation of large effect occurring in the relatively shorter period of separation of sublines of the BALB/cAn strain. Moreover, since we cannot assume the genetic affects on one trait to be independent of those of another, the amount of evidence is more apparent than real.

The values of ulna and ilium lengths being higher than those of skull measurements for both strains suggest that some traits may be more prone to drift than others. However, we cannot rely too heavily on the little evidence presented here. It has previously been shown[1,3] that phenodeviant traits, at least, do not possess markedly different rates of differentiation.

The magnitudes of individual polygenic effects in relation to the mean or the variance of a trait would be desirable information to have. By making observations of the diverging sublines at a single point in time, we were not able to determine the size of the mutant effects or the frequency of their occurrence. Perhaps with an enlarged and more efficiently designed experiment we can provide answers to some of these questions.

Summary

The rates of differentiation of sublines of two inbred strains of mice, C57BL/6 and BALB/cAn, were estimated using as criteria the variances of the subline means of six metric traits of the skeleton. The values were computed as proportions of the genetic variance of a theoretical population of fully inbred strains randomly derived from a panmictic population. The estimates ranged from 0 to .006 in the C57BL/6 strain and from .002 to .013 in the BALB/cAn strain, the rates for the latter strain being consistently higher for all traits. The implications of the magnitudes of these rates for investigations which depend upon the stability of inbred strains of mice have been discussed.

Appendix

Effects of full-sib mating on variance originating by mutation

In the accompanying paper we have made estimates of the rates of spontaneous differenti-

TABLE IV. Rates of genetic divergence per generation in terms of the genetic variance expected between highly inbred strains

	Measurement					
Strain	M	Z	P	F	U	I
C57BL/6	.0015	.0011	.0002	.0000	.0059	.0022
BALB/cAn	.0017	.0032	.0025	.0041	.0127	.0076

TABLE V. Estimates of the number of generations required before measurements in sublines, consisting of 10 mice each, would become significantly different (using five percent level)

	Measurement					
Strain	M	Z	P	F	U	I
C57BL/6	50	93	422	—	13	30
BALB/cAn	45	32	25	19	6	9

ation of sublines of highly inbred strains due to the fixation of newly arising mutations. It is desirable to express these estimates in terms of new genetic variance being added to a non-inbreeding population. We will show here that no conversion of values is necessary, that the rate at which new variation is accumulated each generation in a panmictic population is equivalent to the rate of differentiation of sublines as we have calculated it.

If we start with an isogenic population consisting of several lines maintained by full-sib matings, the variance in the next generation due solely to new mutations, V_{gm}, will be distributed throughout the population. Such variance will be apportioned in the original and subsequent generations as follows (after Wright[5]):

Generation					
	0	1	2	3 ..... n	
Total	1	1	5/4	11/8	$1 + F_n$
Within lines	1	1/2	1/2	3/8	$(1 - F_{n-1})/2$
Between lines	0	1/2	3/4	1	$(1 + 2F_n + F_{n-1})/2$

where F is the inbreeding coefficient.

Since this variance originates in every generation, it is necessary to summate these effects through all subsequent generations, n, which gives us:

Total $\quad (n + 1 + \sum_{i=1}^{n} F_i)\ V_{gm}$

Within lines $(1/2)\,(n + 2 - \sum_{i=1}^{n} F_i + F_n)\ V_{gm}$

Between lines

$$(1/2)\,(n + 3 \sum_{i=1}^{n} F_i - F_n)\ V_{gm} \qquad (1)$$

If sublines arise in such a population, as has occurred in the strains of mice on which this study is based, we must also consider the presence of variance due to mutations occurring prior to the dichotomy but not yet completely fixed or lost by inbreeding. Professor E. R. Dempster has pointed out to me the convenience of the increment of between variance in each generation being half the within variance of the previous generation. If we let V_{wi} be the within variance in the i^{th} generation, we can express (1) as:

$$(1/2) \sum_{i=0}^{n-1} V_{w_i}$$

If two sublines originate from a single line in the s^{th} generation, the between sublines variance, V_{s1} in generation s+1 will then be $(1/2)\,V_{w_s}$ and by generation n:

$$V_{s1} = (1/2)\ (\sum_{i=0}^{n-1} V_{w_i}) - \sum_{i=0}^{s-1} V_{w_i}) \qquad (2)$$

Substituting expression (1) in (2) we get:

$$V_{s1} = (1/2)\ [(n + 3 \sum_{i=1}^{n} F_i - F_n)$$

$$- (s + 3 \sum_{i=1}^{s} F_i - F_s)]\ V_{gm}$$

$$= (1/2)\,(n - s + 3 \sum_{i=s+1}^{n} F_i - F_n + F_s)\ V_{gm}$$

When s becomes large, say greater than 30, so that all values of F beyond F_{s-1} approach unity,

$$V_{s1} = 2d\ V_{gm} \qquad (3)$$

where d = n−s.

The rate of subline divergence in the accompanying paper was computed as:

$$V_{s1}\ /2dV_{go}$$

where V_{go} is the original genetic variance of the panmictic population from which the inbred strains originated. From expression (3) we can show that the rate of subline divergence is equivalent to the rate of accumulation of gentic variance per generation in a panmictic population, expressed in terms of the genetic variance found in a wild panmictic population, that is,

$$V_{s1}\ /2dV_{go} = V_{gm}/V_{go}.$$

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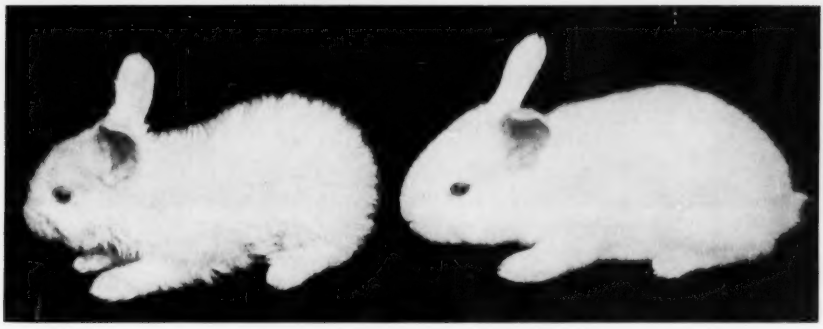
INHERITANCE AND HAIR MORPHOLOGY OF THE "WUZZY" MUTATION IN THE RABBIT

D. D. Crary and P. B. Sawin*



HOMOZYGOUS "WUZZY" AND NORMAL
Figure 5
Wuzzy and normal sibs at 21 days of age.

At least seven mutations of hair structure now have been described in the domestic rabbit as summarized by Sawin[7], Robinson[6], and Nachtsheim[5]. Of these, the morphologies of three, furless (Drapeau[3]), naked (David[2]), and satin (Castle and Law[1]), have been described in considerable detail. Furless is essentially an absense of wool hair due to premature keratinization which affects first the sebaceous glands and then the inner epithelial sheath. Naked resembles furless, but the effect of the gene is exerted in the stratum granulosum and the follicles, being relatively unable to erupt, are reduced in number. Satin is essentially an effect upon the cell structure of the hair, the major expression being a reduction through compaction of the medulla, but with the thickness of the cortex and cuticulum also reduced. The coat presents a smoother surface which is responsible for its greater reflection of light and the greater brilliance of color of these animals.

This paper supplies further details as to gross appearance and microscopic structure of the hair of a fourth hair mutation, "wuzzy"[7], and its inheritance.

Gross Manifestations

The coat of the wuzzy rabbit is normal at birth so far as we have been able to detect. Characteristically, it does not begin to manifest itself until about 10 days, the time the young begin to leave the nest. At this time homozygotes begin to assume the unkempt appearance shown in Figure 5. This is due to a tendency for the tips of the hairs to mat or clump about each other in a peculiar, seemingly twisted fashion which becomes progressively more pronounced as additional hairs become involved (Figure 6). Although the hair does not appear coarse or of enlarged diameter, as is the case in furless (*ff*) or wirehair (*W'hW'h*) ho-

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This investigation was supported in part by research grant B-722 from the National Institute of Neurological Diseases and Blindness, Public Health Service.

NORMAL AND "WUZZY" HAIR SAMPLES

Figure 6

Hair samples from normal and wuzzy showing matted condition of the hair. The appearance of greater length of tufts is artifact arising from the matted state of the hair.

mozygotes, it is characteristically harsh to the touch and often appears to be greasy, tacky, or gritty to the touch. These characteristics indicate some sort of surface irregularity of the hair. Other peculiarities which are fairly common are (1) a greater susceptibility to infections of the eyes, probably due to irritation from adjacent turned-in hairs or hair follicles, which are almost incurable once established, and (2) a tendency to denudation of the hair on and between the forelimbs and neck (Figure 7). The basic cause of this we do not know, unless the roughness of the hair is sufficiently uncomfortable as to induce self plucking.

Microscopic Manifestations

To determine the nature of the surface structure causing the hair to mat, samples were taken from wuzzy and normal sibs at several different ages from two months to maturity, subsequent to the time at which the gross manifestations became apparent. In order to secure clear preparations the study was confined to the pigmentless hair of albinos. Hair samples were immersed in Gomori's modification of Mallory II* overnight, rinsed, and dehydrated in the usual dilutions of alcohol, cleared in xylene and mounted for examination. This resulted in preparations in which normal hair is lightly stained yellow, with nuclei green. In both normal and wuzzy, the root and any hair areas which have been injured in any way tend to be more deeply stained, often including other cellular parts as well as the nuclei. In general, the wuzzy seems to take the stain more easily.

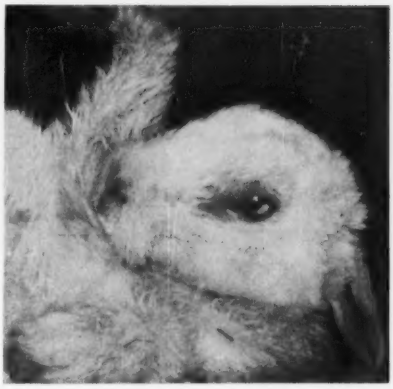
The normal hair (Figure 8*A* and *B*) shows a smooth, thick cuticle and a well defined, orderly arrangement of cells of all three layers, medulla, cortex, and cuticle. In the wuzzy hair (Figure 8*C* and *D*), the cell arrangement is less regular in places. Groups of cells, particularly in the medulla, were found to run together with nuclei compacted and appearing degenerate and thinner. Cortex and cuticle also show obvious irregularities, notably a more sharply defined cuticular spine in groups of cuti-

*Mallory's analine blue-orange G acetic acid solution in which analine blue was replaced with fast green.

cular cells scattered at irregular intervals along the hair.

The most significant feature from the standpoint of the gross matting of the hair is the fact that at about the time of first gross appearance of the character (two to three weeks) the surface of each hair manifests obvious breaks in the cuticular layer which vary from what appear to be simple interruptions of the cell layer to a curling up and separation of this layer, particularly involving the central spine. As the hair begins to mat, the cuticle and spines are found partially separate and assume the appearance of fine, curved, hairlike projections partially separated from their normal position on the surface of the hair (Figure 8*C*).

The previously mentioned greasy, tacky, or gritty feeling of the hair seems to be due to some sort of material, possibly a skin secretion, which collects in small droplets as shown in Figure 8*E*, *F*, and *G*, and which is not manifest in hair of normal rabbits. Its effects in collecting the ruptured cuticular spines and entangling the hair are illustrated in Figure 8*G*.



"WUZZY" MUTANT

Figure 7

The infection of the eyes and denudation of the hair in the region of the forelimb and neck are characteristic of the wuzzy mutant.

Genetics

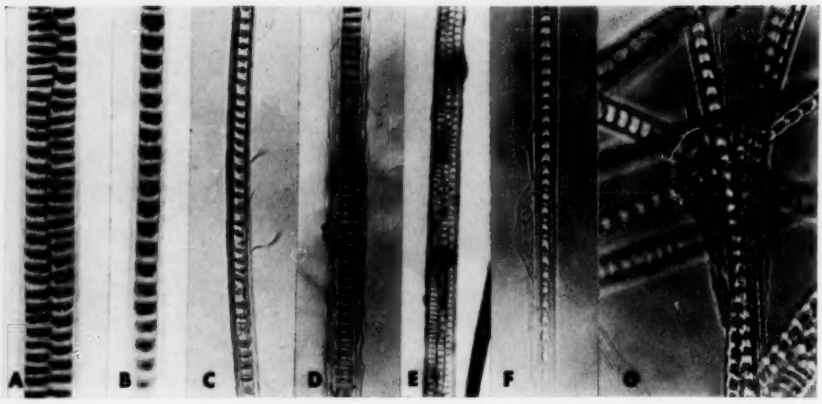
The several generations of offspring sired by the original wuzzy male are shown in Table I. When mated with six unrelated normal females, he produced 33 progeny, all of which were indistinguishable from normal rabbits, indicating that the trait is recessive. When the F_1's were mated back to him and/or to his wuzzy sons, 20 normals and 19 wuzzy progeny resulted, as would be expected if the gene is recessive (mating 2, Table I). From the matings of the F_1's *inter se* (mating 3) 134 progeny were obtained with a ratio slightly deviating from that expected from heterozygous parents. Since no deficiency in wuzzy females was observed among the 134 F_2 offspring and no sex difference in the expression of the primary characters, there is no evidence of any genetic relationship with sex. To date we have been unable to obtain matings from a wuzzy female due to the fact that females have become debilitated, presumably as a result of the above described eye irritation and infection which has either led to death or necessitated early sacrifice.

Discussion

Superficially, the gross manifestation of the "wuzzy" gene on the coat of the rabbit has certain resemblances to those of the "crinkled" gene in the mouse described in considerable detail by Falconer, Fraser, and King[4], and pictured by Silvers and Lane[8]. Both genes manifest a clumping of the hair, balding or denudation of certain regions, and corneal ulceration. In crinkled the clumped appearance comes about through follicular deficiency or absence of certain types of hair. This finds greater expression ventrally, leading to apparent denudation. In wuzzy the clumping is a structural defect of the hair and denudation appears to be a loss of hair, a secondary effect resulting from the grooming of the matted hair. Corneal ulceration of crinkled is a

TABLE I. Summary of wuzzy matings

Matings	Offspring No.	Phenotype Normal Obs.	Normal Exp.	Wuzzy Obs.	Wuzzy Exp.	χ^2	P
1. Wuzzy ♂ × Normal ♀ (8)	33	33	(33)	—	—	—	—
2. F_1 ♀ × Wuzzy ♂ (8)	39	20	(19.5)	19	(19.5)	.0127	90%
3. F_1 *inter se* (23)	134	101	(100.5)	33	(33.5)	.008	94%



NORMAL AND "WUZZY" HAIR

Figure 8

In order from left to right are: *A*—normal secondary guard hair; *B*—normal wool hair; *C*—wuzzy wool hair showing weakened cuticular scales standing out at right angles; *D*—wuzzy wool hair with irregular, fused, or joining medullary cells; *E*—wuzzy primary guard hair with dark staining gelatinous appearing masses on surface of the hair; *F*—wuzzy wool hair with similar gelatinous masses; and *G*—wuzzy hairs entangled or matted by gelatinous mass and cuticular scales. The latter are most clearly in evidence at top and bottom, indicated by arrows. *A* and *B* are under higher magnification than the other photographs.

matter of glandular deficiency whereas in wuzzy, if it is of glandular origin, it seems a matter of over- rather than under-secretion. In addition, sloughing of the cuticular scales provides a further source of irritation. Thus, the two genes actually have little in common etiologically. Whether there are other pleiotropic effects of the wuzzy gene upon other systems we are not prepared at this time to state. Much remains to be learned about the action of the wuzzy gene with respect to both the basic effect in the integument and also its possible pleiotropic effects elsewhere than in the skin.

Summary

A recessive defect of the hair is reported for the domestic rabbit. Gross matted clumping of the hair, ulceration of the eye, and minimal denudation are secondary effects of structural changes in medula, cortex, and particularly of the cuticular scales. Ultimate separation of these scales from the surface of the hair, enhanced by abnormal secretion from the skin, leads to entanglement of the hair, irritation of the eye and skin, and secondarily to localized denudation.

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A MUTATION CAUSING NEONATAL JAUNDICE IN THE HOUSE MOUSE

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HEREDITARY jaundice has been described in the rat[3,6], and the deer mouse[4,5]. The syndromes are similar in both animals and include: neonatal jaundice, anemia, bilirubinemia, reticulocytosis, and splenomegaly. The red blood cells of jaundiced rats are normocytic[6], while those of affected deer mice vary in size and shape[4]. Both diseases are due to the action of recessive genes.

The present article presents the results of some genetic studies and a preliminary description of the effects of a new mutation in inbred strain 129 mice which produces congenital anemia and neonatal jaundice. This lethal mutation is named jaundiced, with the symbol *ja*, and is caused by the action of a single, autosomal, recessive gene with regular manifestation.

Methods

The first jaundiced animals were observed shortly after birth in litters of three pairs of closely related strain 129 mice, and were easily recognized by the yellow coloration of the skin. A colony was established using these pairs, their offspring, and other strain 129 females mated with known carriers (*Jaja*). The presence of jaundiced young indicated that the individuals being tested were carriers, while those producing all normals from a minimum of 10 (later 20) offspring were considered to be non-carriers (*JaJa*).

Each week pregnant animals were placed in separate pens which were checked two or three times daily (except Sunday) for newborn litters. By frequent checking, newborn animals of the *jaja* genotype were found to be anemic at birth and to acquire the yellow color within a few hours. Pale animals found dead in new litters, though most likely of the *jaja* genotype, were not considered as *jaja* animals in the breeding tests to be described.

Since all jaundiced mice die shortly after birth, it was necessary to transplant ovaries from *jaja* newborns into the ovarian capsules of castrated *JaJa* hosts in order to obtain matings involving the *jaja* genotype. In the present case, the jaundiced donors were of the genotype *ccpp* (albino) or $c^{ch}c^{ch}pp$ (pink-eyed chinchilla). Some of the hosts were F_1 DBA $\times$ strain 129 hybrids, and others were fully pigmented strain 129 mice, developed by crossing strains 129 and DBA, followed by repeated backcrosses to strain 129 with selection for the genes *C* and *P*. The hosts, then, were of the genotypes *Cc*, Cc^{ch} or *CC* (pigmented coat) and *Pp* or *PP* (pigmented eyes). By making appropriate matings, offspring of mice with grafted ovaries could be positively identified by coat and eye color as being from the graft or from possible reconstituted ovarian tissue of the host. Further details of the technique of ovarian transplantation and mating systems are described by Stevens[11,13].

Results

1. Genetics

Animals known to be carriers of jaundiced (presumed *Jaja*) were mated *inter se*, with non-carriers (*JaJa*); and both genotypes were mated to normal animals bearing ovarian grafts of the *jaja* genotype. The results of these matings are summarized in Table I.

The matings listed in Table I produced fewer affected offspring than expected on the basis of unit recessive inheritance. This deficiency may be attributed to prenatal and/or neonatal death of affected animals before the onset of the jaundice. Males and females were affected in approximately equal numbers.

Another attempt to determine the nature of

* Roscoe B. Jackson Memorial Laboratory, Bar Harbor Maine. This investigation was supported in part by grant IN-19 from the American Cancer Society and by grant AEC 1800 from the Atomic Energy Commission. The technical assistance of Mrs. Hannah Cunningham is gratefully acknowledged.

the inheritance of jaundiced was made by genetically testing the offspring of the original male carriers (presumed *Jaja*) when mated to normal (*JaJa*) females. These F_1 animals were mated with known carriers (*Jaja*) and the proportion of them having jaundiced offspring was noted. If the genotype of these males producing jaundiced offspring is indeed *Jaja*, then one-half of their offspring when mated with normal females would be expected to be carriers. The results of this test are presented in Table II.

When heterozygous males (*Jaja*) were bred to normal females (*JaJa*), approximately one-half of their male offspring were heterozygous, indicating that the *ja* gene is located on an autosome.

Term fetuses from matings of known heterozygous *Jaja* animals were examined. As shown in Table III, 19 percent of these exhibited a marked pallor and were assumed to be genetically *jaja*. Dead fetuses comprised six percent of the total and were not included in these results. Apparently there is a decreased prenatal survival of *jaja* mice. These data add further support to the hypothesis that congenital anemia and neonatal jaundice in strain 129 mice are produced by a single recessive, autosomal gene.

2. *Pathology*

Examination of 15-day old fetuses resulting from the mating of two heterozygotes (*Jaja*) revealed the presence of pale but not yellow offspring in the expected ratio of one in four. Even at this stage in development the anemia was apparent. The liver and heart were enlarged, while the spleen and other organs appeared normal. Newborn jaundiced mice autopsied six to 12 hours after birth had a marked yellow coloration of the skin and upper portion of the small intestine, but other organs were not discernibly tinted. No additional abnormalities were noted. The lack of gross yellow coloration of the affected fetuses suggests that the end products of red blood cell destruction, if such a process is occurring, are able to pass the placental barrier and are successfully removed via the maternal circulation. Animals examined at two days *postpartum* had spleens and livers nearly twice the size of their non-jaundiced littermates, and cardiac enlargement was also a prominent feature. Histological examination of the livers revealed the presence of numerous small necrotic areas, as well as large numbers of uniformly dispersed hematopoietic foci.

3. *Hematology*

Hematological analyses clearly indicate that jaundiced mice are affected with a severe microcytic anemia. Newborn *jaja* mice, for example, have average erythrocyte counts of 2×10^6 RBC/mm^3 while normal sibs average 4×10^6 RBC/mm^3. Mean cell volumes range from 40-70 cubic microns for *jaja* at birth as compared with 100-120 cubic microns for non-affected sibs. The anemia is further charac-

TABLE I. Breeding tests involving carriers (presumed *Jaja*), non-carriers (*JaJa*), and mice bearing *jaja* ovarian grafts

Hypothetical genotype of parents ♀ ♂	No. offspring born	No. jaundiced	% jaundiced	χ^2	P
JaJa × *Jaja*	718	0			
Jaja × *JaJa*	346	0			
Jaja × *Jaja*	1555	293	19	31 for 3:1	.01
jaja ovarian graft × *JaJa*	6	0	0		
jaja ovarian graft × *Jaja*	106	31	29	18 for 1:1	.01

TABLE II. Genetic test of offspring of *JaJa* × *Jaja* matings when crossed with known carriers (*Jaja*)

Parents ♀ ♂	No. tested	No. carriers ♀	No. carriers ♂	No. non-carriers ♀	No. non-carriers ♂	χ^2 for 1:1	P
Ja— × *Jaja*	71	24	19	13	15	3.106	.08

TABLE III. Observations of term fetuses resulting from matings of *Jaja* × *Jaja*

No. fetuses observed	No. anemic	% anemic	No. dead	% dead	χ^2 for 3:1	P
78	14	19	5	6	1.320	.25

terized by anisocytosis, poikilocytosis, reticulocytosis, hypochromia, erythroblastosis, and polychromatophilia. In addition, many of the erythrocytes show basophilic stippling ,and contain other inclusion bodies. The number of siderocytes is not increased above the level characteristic of mice of this age[2]. Schistocytes are commonly encountered in smears but target cells and spherocytes have not been observed. The picture then is one of severe anemia in which considerable erythrocyte destruction is occurring. In an apparent attempt to compensate for the paucity of red blood cells many immature cells are released from the hemotopoietic organs. These cells, consisting of members of the erythroid series not commonly encountered in the circulation as well as myeloid elements give the peripheral blood its characteristic picture. A more detailed hematological account will appear elsewhere.

Congenital conditions somewhat similar to jaundiced have been encountered in the mouse and other rodents. A comparison of jaundiced with these abnormalities is presented in Table IV.

Discussion

The breeding data presented above suggests that congenital anemia and neonatal jaundice in strain 129 mice are caused by a recessive gene. Phenotypically normal carriers (*Jaja*) when mated *inter se* produced offspring, 19 percent of which were jaundiced. When carriers were mated with normal animals, none of their offspring were jaundiced, but approximately one-half of them were heterozygous *Jaja* as revealed by mating them with known carriers. Matings of animals bearing homozygous *jaja* ovaries with normal mice produced 0 percent affected offspring, and with carriers, 29 percent.

The proportional deficiency of observed vs. expected jaundiced offspring may be a result of early mortality of genetically jaundiced young before they could be classified phenotypically. Animals born dead, even though anemic, were not classified as *jaja* since they did not have yellow skin coloration. Some of these might have become yellow had they survived a few hours. A deficiency of *jaja* term fetuses taken by Caesarean section indicates

TABLE IV. A comparison of jaundiced with similar hematological conditions in rodents

	Mus			*Peromyscus*	*Rattus*
	Jaundiced	Flexed-tail anemia[2]	Diminutive[12]	Hereditary spherocytosis[4,5]	Hereditary acholuric jaundice[1,3,6,10]
Inheritance	autosomal recessive lethal	autosomal recessive	autosomal recessive	autosomal recessive	autosomal recessive[3]
Type of neonatal anemia	severe microcytic hypochromic	mild transitory normocytic hypochromic	severe permanent macrocytic	mild spherocytic	mild normocytic[6] microcytic[3] normochromic[6,10]
Neonatal jaundice	present	absent	absent	present	present[3,6]
Splenomegaly	present	not reported	not reported	present	present[3] microsplenic[6]
Siderocytosis	absent	present	absent	not reported	not reported
Erythroblastosis	present	not reported	not reported	not reported	not reported
Reticulocytosis	present	present	not reported	present	present[3,6] absent[10]
Anisocytosis	present	not reported	not reported	present	not reported
Osmotic fragility of RBC	not determined	normal	not reported	increased	increased[3] normal[6]
Growth	retarded	retarded	retarded	not reported	retarded
Skeletal abnormalities	none observed	present	present	not reported	not reported
Defect	not known	abnormal liver hematopoiesis	not known	production of abnormal RBC	reduced glucuronide formation[1]

that there is a selective prenatal mortality of affected animals. There is little variation in the severity of the jaundiced condition, making phenotypic misclassification an unlikely explanation for the deficiency in numbers of *jaja* animals. Furthermore, none of the phenotypically normal carriers produced abnormal offspring in proportions higher than expected for heterozygotes. The possibility that the *jaja* genotype exhibits incomplete penetrance seems unlikely.

The applicability of the method of ovarian transplantation for recovering germ cells of lethal genotypes has been demonstrated in the case of the mutation dystrophia muscularis, *dydy*, a primary myopathy which also occurred in strain 129 mice[13]. The use of ovarian grafts provided the only opportunity of utilizing gametes of the lethal *jaja* genotype. When females with *jaja* ovarian grafts were mated with either *JaJa* or *Jaja* males, they produced phenotypically normal male offspring which were heterozygous for jaundiced. This evidence demonstrates that the jaundiced gene is not located on the sex chromosome. Another breeding test, where *Jaja* males were derived from *JaJa* females bred with *Jaja* males further supports the conclusion that the *ja* allele is located on an autosome.

Hematologically, the gene produces a microcytic, non-siderocytic anemia unlike flexed-tail anemia which is normocytic and siderocytic[2]. Further evidence suggesting that these two genes, *ja* and *f*, are different is provided by two *jaja* mice which were kept alive for six months by daily blood transfusions. Neither of these showed tail-kinking or belly-spotting characteristic of flexed. Other congenital anemias of the mouse also differ from jaundiced. Diminutive (*dm*) produces abnormalities of the axial skeleton and a permanent macrocytic anemia[12]; dominant spotting (*W*, W^v, W^j) produces distinctive coat color effects and macrocytic anemia[2, 7, 8]; and steel (*Sl*) exhibits dilution of pigmentation[9]. Similarly the characteristics of jaundiced are sufficiently different from hereditary spherocytosis (*sp*) in *Peromyscus*[4, 5] and hereditary acholuric jaundice (*n*) in rats[1, 3, 6, 10] to constitute a new and unique hematological abnormality.

The question of blood group incompatibility may be raised as a possible cause of this condition. The information available at this time makes this possibility seem unlikely, for several reasons: 1) the recessive mode of inheritance; 2) the fact that the mixture of maternal and *jaja* blood *in vitro* fails to show the presence of maternal hemagglutinins; and 3) no sensitization process is seen, i.e. litter size and proportion of affected offspring, resulting from the mating of two heterozygotes, does not vary with litter number as would be expected if there were increasing prenatal mortality. The proportion of affected mice does not increase in successive litters as might be expected if there were a fetal-maternal incompatibility. Accordingly, the erythroblastosis characteristic of jaundiced is probably not the result of an antigen-antibody reaction similar to that encountered in human erythroblastosis fetalis.

We may conclude then that the jaundiced condition in strain 129 mice is due to the action of a single lethal, autosomal, recessive gene with regular manifestation and is characterized by microcytic anemia, neonatal jaundice and splenomegaly.

Summary

A new hematological mutant called jaundiced, with symbol *ja*, characterized by microcytic anemia, neonatal jaundice, and splenomegaly is described.

The disease, resulting from the action of a Mendelian recessive autosomal gene, is invariably lethal in the homozygous condition. Heterozygotes are viable and fertile but visibly indistinguishable from homozygous normals.

Jaundiced is considered to be different from other hematological mutants such as flexed-tail, steel, dominant spotting, diminutive, hereditary spherocytosis, or hereditary acholuric jaundice.

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HEPATIC FUSION, A NEW GENE IN LINKAGE GROUP I OF THE MOUSE

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DURING routine autopsy of mice belonging to the MA/MyJax inbred strain, a distinct fusion was noted between the left portion of the median liver lobe and the posteriorly adjacent left lateral lobe. Examination also revealed the fusion in MA strain animals, the parent strain from which MA/My stock was derived[3]. All animals examined from these strains have shown some degree of this trait. The condition is detectable in the 15-day fetus, and has probably reached its final degree of development before the birth of the animal. Histologically the condition suggests a failure of normal lobation rather than a mutual attachment of the lobes after they were formed. The term fusion is retained on the basis of the gross appearance as a convenient descriptive term.

The degree of fusion varies from an amount barely distinguishable from the normal liver pattern, to a grossly complete fusion of the entire normal area of contact between the lobes involved. For comparative measurement, the entire range of fusion was divided into five degrees. The first degree represents animals with a fusion involving up to one-fifth of the normal area of contact between the lobes concerned; the second degree represents animals with liver lobes between one-fifth and two-fifths fused; etc. . .; degree five includes those animals with lobes four-fifths to completely fused. In actual laboratory procedure the area fused was estimated from gross observation at the time of autopsy. An illustration of the normal liver pattern, and of each of the five degrees of fusion, is found in Figure 9. No evidence of a similar fusion of the liver lobes has been found in any other of the several inbred strains of mice routinely autopsied in this laboratory. Few reports of variation in the pattern of liver lobation in mice were found in the literature; these are often in the direction of fission rather than fusion[1, 4].

Genetics

Preliminary crosses of MA/My animals, genetically albino (*cc*), with strains DBA and C3H produced F_1 progeny all of which were colored and had a normal liver pattern. The F_1 animals were mated *inter se* to give an F_2 generation, and also backcrossed to strain MA/My. The results of these crosses are shown in Table I.

It will be seen that five of 37 F_2 mice and 41 of 72 backcross mice were classified as fused. It is also evident that there is a deficiency of the classes showing a recombination with albinism. These results can be explained by assuming that hepatic fusion is caused by a recessive gene linked with albinism. However, since the variability of the fused trait led to some uncertainty in classification, further tests appeared desirable.

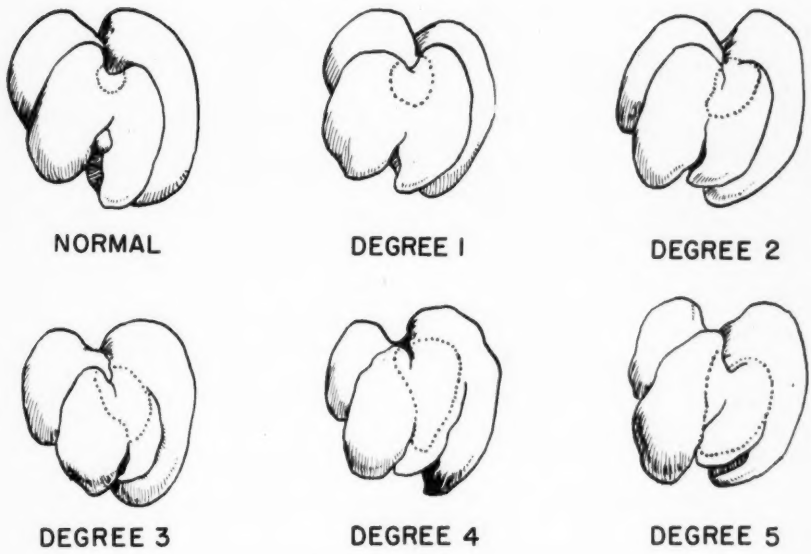
The stocks selected for the tests were MA/My and 129. The latter carries the linked genes c^{ch}, an albino allele, and *p*. The gene assumed to determine the fused condition was designated *hf* (hepatic fusion). The testing

TABLE I. Phenotype and number of F_2 and backcross progeny from crosses of MA/My with DBA and C3H

Phenotype	F_2 progeny		Backcross progeny	
	No. observed	No. expected*	No. observed	No. expected*
Colored non-fused	30	20.9	30	18
Colored fused	0	6.9	7†	18
Albino non-fused	2	6.9	1	18
Albino fused	5	2.3	34†	18

*Assuming two independent genes
†6 colored and 2 albino animals very weakly fused

* Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The author is very much indebted to Dr. G. D. Snell, for his valuable assistance in planning the work and in writing this report. The abnormal liver lobation was first noticed by Fredonna Hodgkins and Dorothy Chapman, research assistants at this laboratory. The author intends to submit a report of the work described here as a thesis in partial fulfillment of the requirements for the Master's Degree at the University of Maine.



LIVER LOBATION

Figure 9

Normal pattern of liver lobation from a strain 129 mouse. Typical fused liver patterns from MA/My animals. The five degrees of fusion are represented. The area within the dotted lines represents the area of fusion between the median lobe and the left lateral lobe.

program consisted of the sequence of MA/My and strain 129 matings outlined in Figure 10.

The livers of all F_1 animals were examined either at weaning or following their use as breeders and found free of the fused trait. However, as we shall point out later, heterozygous mice on a predominately MA/My genetic background sometimes show a low degree of fusion, so that the trait is not always completely recessive.

Sixty-eight offspring from the backcross mating $F_1 \times 129$ were separated on the basis of coat color into the four possible genotypes involving c^{ch} and *p*. There were 11 recognizable crossovers. All 68 backcross offspring were then mated with MA/My animals in a progeny test to detect *hf* heterozygotes. The number of progeny examined from each backcross animal was 10 or more except in five cases where fewer than 10 were raised. One animal classified as a crossover was tested by eight progeny; all other crossovers were tested by 12 to 31 progeny each.

The results are shown in Table II. The test generation mice in this table are divided into $c^{ch}c$ and *cc* groups, and into fusion (degrees 2-5), indefinite (degree 1), and non-fusion groups. Of the 32 phenotypically $c^{ch}c^{ch}$ backcross mice, 31 (last two lines in the table) produced 433 test offspring of which six were classified as fused (all of degree 2, and no more than two of the fused offspring from any one backcross mouse), 28 as indefinite, and 399 as not fused. In contrast to this, one $c^{ch}c^{ch}$ backcross mouse, ♀55, a known crossover between *c* and *p*, produced 14 fused offspring, (three of which were of fusion degree 2, and 11 of the higher degrees 3-5), and nine non-fused. This mouse was classified as c^{ch} *hf* $+/c^{ch}$ + *p*, and hence the product of a crossover in the interval *c-hf*. All other $c^{ch}c^{ch}$ were interpreted as being homozygous non-fusion, even though 34 of their 433 offspring, all of which must have been *Hfhf*, showed a low grade of the trait. We will comment later on these exceptional animals.

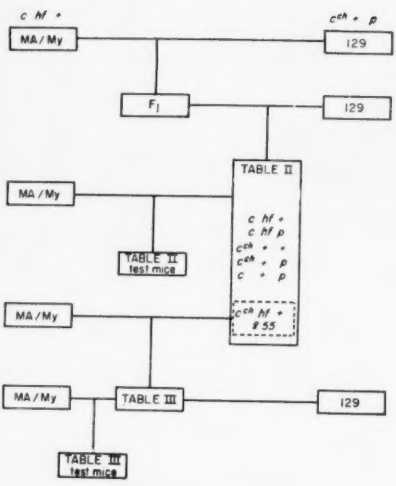
The same unexpected types probably occurred also among the offspring of the 36 backcross mice phenotypically cc^{ch}, though the situation here is more complicated. All these mice threw both *cc* and $c^{ch}c$ test generation offspring in approximately equal numbers, most of the former being fused, and most of the latter non-fused. The first of these categories is the most informative. Thirty-five of the 36 cc^{ch} backcross mice (first two lines of Table II) threw 280 test generation mice phenotypically *cc*. With regard to liver structure, 226 of these were classified as fusion, 33 as indefinite, and 21 as non-fusion.

Not more than three of these non-fusion came from any one parent. On the basis of these results, the 35 parents were classified as *c hf*/c^{ch} +. Some of the *cc* non-fused and $c^{ch}c$ fused among their test generation offspring were probably the result of crossing over between *c* and *hf*, but some of the former may have been due to incomplete penetrance of *hf*, and some of the latter an expression of *hf* in the heterozygotes. One of the 36 cc^{ch} backcross mice, ♂4, a known crossover between *c* and *p*, threw, besides seven $c^{ch}c$ non-fusion and one $c^{ch}c$ indefinite, six *cc* all non-fusion. This mouse bred like a non-fusion homozygote, genotype *c* + *p*/c^{ch} + *p*, and hence a crossover between *c* and *hf*.

To further confirm the classification of the backcross animals, ♀55, presumed to be c^{ch} *hf* +/c^{ch} + *p*, and hence the product of a crossover between *c* and *hf*, was subjected to additional tests. A mating was made to MA/My, yielding 16 offspring. These were tested for *p* by mating to 129, and for *hf* by mating to MA/My and by subsequent autopsy. The last named tests gave concordant results. The data, summarized in Table III, are consistent with the presumed genotype of ♀55. Thirteen of the 16 animals are non-crossover types, three are crossovers between *hf* and *p*.

At the time the data were summarized some doubt arose concerning the proper identity of one of the backcross offspring phenotypically classified in the mouse room records as heterozygous chinchilla, non-pink eye (*c* . . +/c^{ch} + *p*). Because this animal was no longer available, the record of its offspring from the testcross mating to MA/My was reexamined. This mating yielded no albinos in a total of 13 non-fused progeny. It is thus highly probable that the animal in question was misclassified and was actually of genotype c^{ch} + +/c^{ch} + *p*, and is so placed in Table II.

We may conclude from the data in Table II



BREEDING PLAN

Figure 10

Breeding plan with reference to text tables.

that *hf* lies between *c* and *p*. Of the 68 backcross animals 2.9 percent represented crossovers between *c* and *hf*, 13.2 percent between *hf* and *p*, and 16.1 percent between *c* and *p*. There were no double crossovers. Table III adds three more crossovers in the *hf*-*p* interval out of 16 animals produced by ♀55, raising the total for this interval to 14.3 percent. In the case of four backcross mice in Table II, including one crossover, the sex of the heterozygous parent is unknown. Omitting these

TABLE II. Result of F_1 × 129 backcross, *c hf* +/c^{ch} + *p* × c^{ch} + *p*/c^{ch} + *p*. Offspring classified for *hf* by progeny test

Backcross mice		Offspring of backcross mice by MA/My (*c hf* +/*c hf* +)					
		cc			$c^{ch}c$		
Presumed genotype	Number	Fusion	Indef.	Non-fusion	Fusion	Indef.	Non-fusion
c hf +/c^{ch} + *p*	30	172	27	14	9	13	201
c hf p/c^{ch} + *p*	5	54	6	7	1	1	43
c + +/c^{ch} + *p*	0	—	—	—	—	—	—
c + *p*/c^{ch} + *p*	1	0	0	6	0	1	7
c^{ch} *hf* +/c^{ch} + *p*	1	—	—	—	14	0	9
c^{ch} *hf p*/c^{ch} + *p*	0	—	—	—	—	—	—
c^{ch} + +/c^{ch} + *p*	4	—	—	—	0	6	47
c^{ch} + *p*/c^{ch} + *p*	27	—	—	—	6	22	352

Total number of animals = 68
Crossover percent, *c*-*hf* = 2.9 ± 1.4
Crossover percent, *hf*-*p* = 13.2 ± 2.8
Crossover percent, *c*-*p* = 16.1 ± 3.1

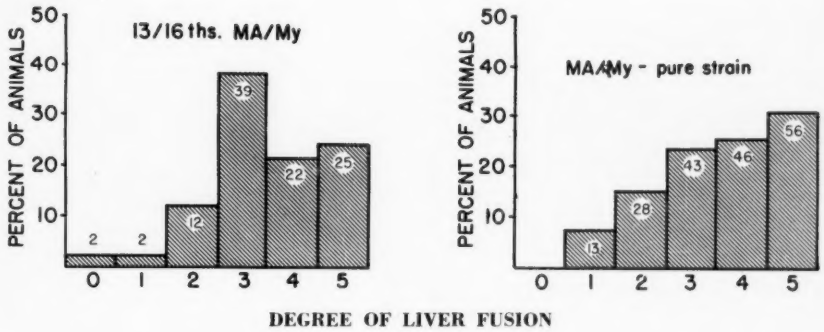


Figure 11

Comparison of degree of liver fusion in pure strain MA/My animals with the group having a genetic background of 13/16ths MA/My from Table IV, line 4. Figures in columns represent number of animals.

four mice, the crossover percent according to the sex of the heterozygous parent becomes: *c-hf* interval in males 4.0 percent, in females 2.6 percent; *hf-p* interval in males 12.0 percent, in females 14.5 percent; *c-p* interval in males 16.0 percent, in females 17.1 percent. The values for the *c-p* interval are in satisfactory agreement with previously published data[2].

We have already noted the wide variations in the expression of hepatic fusion. Table IV presents combined figures for certain of the test generation mice of Tables II and III selected to show the extent of this variation in various genotypes. The MA/My genetic background is probably a factor in this variability. The mice of Table II are from a cross such that 10/16ths of their genes were derived from strain MA/My, and the mice of Table III from a cross such that 13/16ths of their genes came from this source.

The animals in the first line of Table IV are of the genotype + *hf*, and 10/16ths MA/My background. Of 380 such mice there were, as we have already noted, six classified as fusion (all of degree 2) and 22 indefinite. Since the classification was based on gross inspection only, it is of course possible that some of the animals were given too high a grade; nevertheless it would seem unquestionable that in animals with this much MA/My background there can be some degree of hepatic fusion in animals not homozygous for the hepatic fusion gene. Whether a single dose of the *hf* gene is a factor in this is uncertain. Likewise line 4 of Table IV shows that *hfhf* animals, 13/16ths MA/My background, can include a low percentage of non-fused (in this instance, two out of 102) and indefinite (two out of 102 individuals).

Lines 2, 3, 5, and 6 of Table IV show animals from matings of the backcross type, *c hf*/c^{ch}+ × *c hf*/*c hf*, taken from the first two lines of Table II and the last two lines of Table III. The problem here is to separate the crossovers from the mice which, because of phenotypic variation, are misclassified. Lines 2 and 3 are $c^{ch}c$ animals which are mostly +*hf* but also include *hfhf* crossovers. These can be compared with the animals of line 1 which are all +*hf*. The most accurate comparison is provided by lines 1 and 2 animals which are both 10/16ths MA/My. It will be seen at once that there are relatively more fused in line 2, and these may be presumed to represent the crossovers. We may arrive at an approximation of the number of crossovers

TABLE III. Result of mating ♀55 (presumed to be c^{ch} *hf* +/c^{ch} + *p*) × MA/My (*c hf* +/*c hf* +). Offspring classified for *p* by mating to 129, and for *hf* by mating to MA/My

Offspring ♀55 × MA/My		Offspring (♀55 × MA/My) × MA/My					
		cc			$c^{ch}c$		
Presumed genotype	Number	Fusion	Indef.	Non-fusion	Fusion	Indef.	Non-fusion
c^{ch} *hf* +/*c hf* +	6	48	0	2	38	2	0
c^{ch} *hf p*/*c hf* +	1	2	0	0	10	0	0
c^{ch} + +/*c hf* +	2	15	2	0	0	0	7
c^{ch} + *p*/*c hf* +	7	46	1	3	7	1	48

Crossover percent, *hf-p* = 18.8 ± 9.7

by transferring to the non-fused category in line 2 all indefinites and a fraction of the fused, proportional to their incidence in line 1. This gives us, out of 268 mice in line 2, six animals, or 2.2 percent, presumed to be genetically fused and hence true crossovers in the *c-hf* region. This agrees very well with the value of 2.9 percent calculated from the data in Table I.

If the same sort of correction is applied to the *cc* backcross animals in line 5, using the homozygous *hfhf* in line 4, also 13/16ths MA/My, to derive the proportion of normal overlaps, we arrive at 5.4 as the percent crossing-over between *c* and *hf*. These figures are subject to a large element of uncertainty, but they are definitely compatible with the crossover values already arrived at.

The data in Table IV are also informative relevant to the effect of MA/My genes other than *hf* on the appearance of fused animals. The 548 animals in lines 2 and 6 were from a backcross-type mating and the offspring were 10/16ths MA/My. The 130 animals in lines 3 and 5 were from a similar mating except that the offspring were 13/16ths MA/My. It will be seen that in the second named group, the animals are all displaced in the fusion direction, e.g., 11.1 as compared with 3.7 percent fusion among the $c^{ch}c$ mice, and 9.1 as compared with 8.1 percent fusion among the *cc* mice.

Additional evidence as to the effect of the genetic background of strain MA/My on hepatic fusion is provided by a comparison of the various grades of fusion in pure MA/My animals as compared with the 13/16ths MA/My animals shown in line 4 of Table IV. Both groups are homozygous *hfhf*. Such a comparison is made in Figure 11. It will be seen that just as the expression of fusion is more pronounced in 13/16ths MA/My animals than in 10/16ths ones, so it is more pronounced in pure MA/My's than in those that have received 13/16ths of their genes from this strain. Evidently besides the one major gene for hepatic fusion, *hf*, the MA/My strain carries one or more other genes favorable to the expression of the trait. The evidence does not show whether these genes act only in the presence of one or more doses of *hf*, or whether they can produce some degree of fusion in the absence of this gene.

Summary

A gene, designated *hf* (hepatic fusion), is important in the production of an apparent fusion of the median and left lateral liver lobes of the MA and MA/My strains of inbred mice.

Hf is a member of linkage group I, lying between *c* and *p*. In crosses where the genotype was verified by progeny test, the *hf* locus was found at 2.9 ± 1.4 crossover units from albinism, *c*, and 14.3 ± 4.2 crossover units from pink eye, *p*.

The fusion is variable in degree from an amount scarcely distinguishable from the normal liver pattern, to a grossly complete fusion between the lobes involved.

Increase in mean degree of fusion for a group is coincident with an increase in MA/My genetic background.

It is probable that factors other than *hf* are also influential in MA and MA/My liver fusion.

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TABLE IV. Combined figures for test generation mice of Tables II and III, showing variation in expression of fusion in *+hf* and *hfhf* mice, and the probable occurrence of crossovers in the *c-hf* interval

Parents: *c hf/c hf* ×	Fraction MA/My genes	Albino genotype	Hepatic fusion genotype	Fusion	Indef.	Non-fusion	Total
c^{ch} +/c^{ch} +	10/16	$c^{ch}c$	*+hf*	6	22	352	380
c hf/c^{ch} +	10/16	"	*+hf* and crossovers *hfhf*	10	14	244	268
"	13/16	"	"	7	1	55	63
c^{ch} *hf*/*c hf*	13/16	$c^{ch}c$ + *cc*	*hfhf*	98	2	2	102
c hf/c^{ch} +	13/16	*cc*	*hfhf* and crossovers *+hf*	61	3	3	67
"	10/16	"	"	226	33	21	280

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